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7th

Asian Fisheries Forum 04

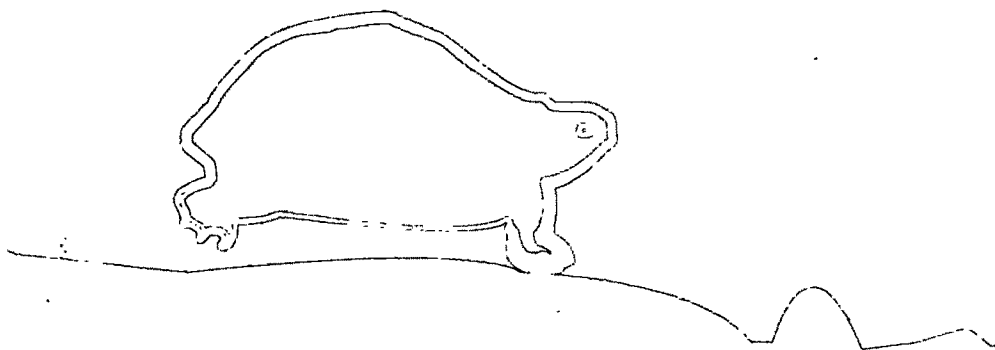
THE TRIENNIAL MEETING OF THE ASIAN FISHERIES SOCIETY

30 November - 4 December 2004 **PENANG, MALAYSIA**

ABSTRACTS

- **Increasing Aquaculture Productivity**
- **Promoting Environmental Friendly Aquaculture**
- **Value Added Products**
- **Enhancing the Ornamental Fish Industry**
- **SS Advances in Shrimp Biotechnology II**
- **SS Advances in Seaweed Cultivation and Utilization in Asia**
- **SS Mariculture in the Asia Pacific Region :**
Recent Developments and New Challenges
- **SS Technology Needs and Prospects for Asian Aquaculture**
- **SS Production in Aquatic Peri-urban Systems in Southeast Asia**
- **SS Aquatic Ecosystem Health Management**
- **SS Improve Resource Use Efficiency in Asian Integrated Pond-dike Systems**

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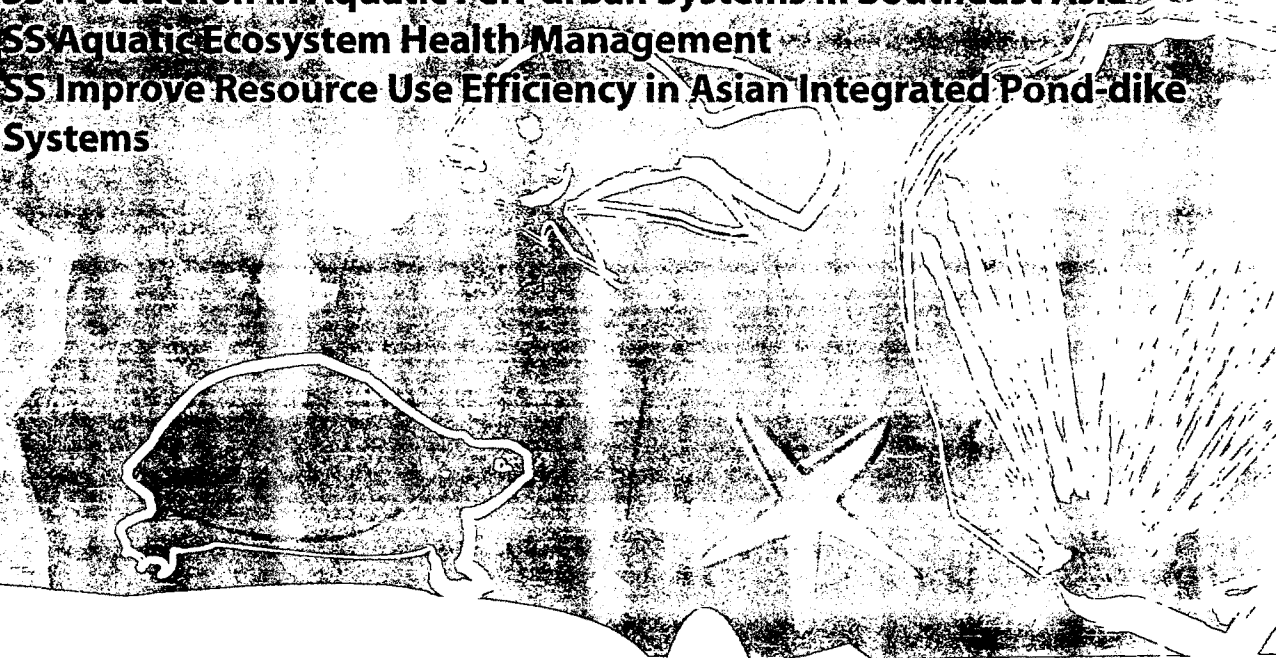


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FOREWORD

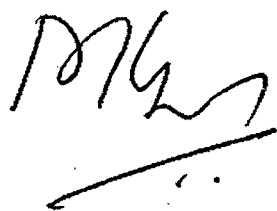
The 7th Asian Fisheries Forum with the theme "New Dimensions and Challenges in Asian Fisheries in the 21st Century" comes at a time when the fisheries sector is facing challenges of declining production from capture fisheries and increasing demand for fish not only in Asia, but globally. Asian countries have been playing a pivotal role in global fish production and contributing to nutritional security of people especially in developing countries.

The Forum has attracted a large number of abstracts indicating the importance the scientists from the region and globally accord to the triennial forum of the Asian Fisheries Society. However, due to limited time during the Forum, it was not possible to accommodate all submissions for oral presentation. Many of the submissions were received very late much after the closing date for submission of abstracts. The Scientific Committee tried its best to include all the papers received in spite of their being received very late and managed to complete this Book of Abstracts on time due to the tireless efforts of many people in the Local Organizing Committee.

Unlike in the previous fora, 11 special symposia dealing with topics of importance covering both fisheries and aquaculture are being organized during the Forum. Presentations in these symposia are expected to address the constraints that deter the sustainable management and development of aquatic resources. There are plans to publish the proceedings of many of these special symposia in refereed journals.

On behalf of the Scientific Committee, I would like to thank all those who have given their time for reviewing/editing the abstracts received, chairing the various sessions and for convening the special symposia.

I wish all the participants productive four-day meetings.



MODADUGU V GUPTA
Chair, Science Committee

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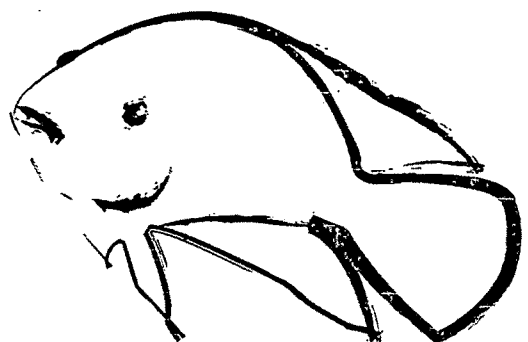
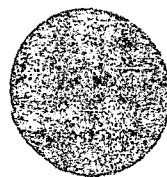
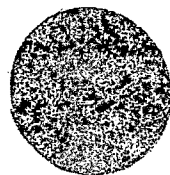
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Increasing Aquaculture Productivity



**EFFECT OF *Squilla* MEAL INCORPORATED DIET ON THE GROWTH AND NUTRITIVE
VALUE OF *Labeo rohita***

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In India, fresh water aquaculture is mainly carp-based, which accounts for about 89% of total aquaculture production. Over the last decade, fish farming has been fast developing from the traditional extensive system to the semi-intensive and intensive culture systems, by increasing the stocking density. As the stocking density exceeds the natural carrying capacity, the dependence shifts from natural food to nutritionally favorable protein rich artificial food to achieve optimum growth and increased production.

The objective of the present study was to formulate and evaluate such artificial feeds by incorporating cost effective animal protein. Accordingly, it has been proposed to test the effect of *Squilla* meal on the growth and nutritive value of the fingerlings of *Labeo rohita*, an indigenous major carp, cultured widely in the state of Andhra Pradesh. Diets of 35% and 40% protein content have been formulated by incorporating *Squilla* powder in the traditional feed of rice bran and groundnut oil cake mixture. A control diet of 35% protein has also been prepared with the traditional feed. Pellets of all these diets, thus formulated, have been prepared in the laboratory with the help of a pellet mill and were dried in a hot air oven with blower. The dry pellets of these artificial diets have been used to feed the experimental fingerlings of *L. rohita* to study the growth, FCR, PER and apparent protein digestibility. The experiments were conducted for 90 days in the laboratory conditions. Preliminary observations revealed that the performance of both the formulated diets is superior to that of the control diet. However, from among the two formulated diets with *Squilla* meal, the one with 35% protein performed better in every respect. Proximate analyses of all the three diets and fish flesh have been carried out to evaluate the relative performance of the two animal protein incorporated artificial diets. The details are dealt in the present paper.

SOYBEAN MEAL SUPPLEMENTED WITH THREE SELECTED AMINO ACIDS IN DIETS FOR *Mystus nemurus* FINGERLINGS UNDER WATER RECIRCULATING SYSTEM

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Seven types of isonitrogenous (42% protein) diets having fish meal with soybean meal (Control diet) and different proportion of soybean meal supplemented with three selected indispensable crystalline amino acids (DL-Lysine, D-Methionine and D-Threonine) were used to determine their suitability as feed for tropical fresh water catfish *Mystus nemurus* fingerlings under small scale water recirculating system {A total of 21 glass tanks (35-L of water) were used in the recirculating aquacultural system; which were in three rows. Three fiberglass elevated filter tanks (0.71m long, 0.53m wide and 0.38m high) were connected in the system that functioned as a trickling biological filter. An airlift pump was arranged in each glass tank to maintain water flow to the collector tank from which water was lifted to the elevated filter tank.}. Casein was added to make up 42% protein. Feed was given twice (930h and 2230h) a day to satiation. The uneaten feed was estimated. The study showed that *M. nemurus* fed with the control diet (D₁) with 25.44% FM and 50% SSBM had the highest growth performances. The species fed the diet with 57% SSBM (D₃) with the selected amino acids had the second highest growth performances. However both treatments (D₁ and D₃) showed no significant differences ($P>0.05$) on the basis of weight gain (%), FCR, SGR, PER, yield and survival (Table 1).

Amino acid profiles of whole-body *M. nemurus* fingerlings at different feeding trials were determined to provide baseline information on the amino acid requirements of the fresh water catfish. The essential amino acid (A/E) ratio were calculated and found to be a possible method for evaluating the *M. nemurus* diet, as a superior ratio indicates superior production, which are not included in this presentation.

TABLE 1: Effect of different percentages of soybean meal on weight gain and protein utilization by *M. nemurus* fingerlings^{1,2}

Treatment	Initial Weight (g)	Weight gain		SGR (% per day)	FCR	PER	Yield (kg/m ³)	Survival (%)
		(g)	(%)					
D ₁	4.46±0.27	7.03±0.40 ^c	158.41±18.08 ^c	1.76±0.13 ^a	0.99±0.07 ^d	2.44±0.15 ^a	4.21±0.16 ^a	79.05±1.09 ^a
D ₂	4.43±0.11	5.25±0.19 ^b	118.67±6.94 ^b	1.45±0.06 ^{bc}	1.34±0.04 ^{abc}	1.67±0.03 ^{cd}	2.85±0.48 ^b	76.19±5.82 ^a
D ₃	4.20±0.23	6.42±0.28 ^c	152.97±11.43 ^c	1.72±0.08 ^{ab}	1.05±0.08 ^{cd}	2.19±0.14 ^{ab}	4.02±0.50 ^a	81.67±4.36 ^a
D ₄	4.55±0.22	5.17±0.17 ^b	114.20±8.89 ^{ab}	1.41±0.08 ^c	1.10±0.02 ^{bcd}	2.08±0.04 ^{bc}	2.93±0.07 ^b	76.43±0.71 ^a
D ₅	4.38±0.04	4.99±0.85 ^b	113.88±18.43 ^{ab}	1.40±0.16 ^c	1.11±0.09 ^{bcd}	2.04±0.20 ^{bc}	2.88±0.59 ^b	79.76±1.80 ^a
D ₆	4.69±0.55	4.37±0.77 ^{ab}	95.26±26.30 ^{ab}	1.23±0.26 ^c	1.34±0.35 ^{ab}	1.74±0.50 ^{cd}	2.43±0.38 ^b	78.57±1.89 ^a
D ₇	4.36±0.23	3.86±0.50 ^a	88.79±13.38 ^a	1.17±0.13 ^c	1.37±0.03 ^a	1.66±0.06 ^d	1.49±0.13 ^c	67.86±5.39 ^b

¹ Mean ± s.d. of three replications. Treatment means within columns with the same superscript are not statistically different ($P>0.05$).

² FCR, feed conversion ratio; SGR, specific growth rate; PER, protein efficiency ratio.

DIETARY ASCORBIC ACID IMPROVES GROWTH AND NON-SPECIFIC IMMUNE RESPONSES OF GROUPER *Epinephelus malabaricus* CHALLENGED WITH *Vibrio carchariae*

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An 8 week feeding trial was conducted to estimate the adequate dietary L-ascorbic acid (AA) requirement for growth of juvenile grouper and to elucidate its effect on non-specific immune responses of grouper when challenged with *Vibrio carchariae*. AA supplemented at 20, 50, 80, 150, 250, 400 and 800 mg/kg diet in the basal diet providing 3, 14, 27, 46, 76, 135 and 288 AA mg /kg diet, respectively. Basal diet without AA supplementation was included as control. There were total of eight experimental diets. Each diet was fed to triplicate groups of grouper (mean weight 7.58 ± 0.05 g).

Fish fed diets supplemented with ≥ 46 mg AA had significantly ($P < 0.05$) greater weight gain (WG) and feed efficiency (FE) than fish fed diets with ≤ 14 mg AA/kg. After the final weighing, ten fish were randomly taken from each aquarium and injected with *Vibrio carchariae* (2.53×10^7 CFU/g grouper body weight). Mortality was recorded daily for 3 days and non-specific immune parameters were monitored for the remaining fish. Higher cumulative mortality was observed in fish fed diet supplemented with 3 mg AA or the AA deprived control diet. Fish fed diet with 288 mg AA /kg had higher respiratory burst activity (superoxide O_2^-) of head kidney leukocyte and alternative complement activity than fish fed the control diet or diets with ≤ 76 mg AA /kg. Lysozyme activity was higher in fish fed diets with ≥ 76 mg AA than fish fed diets with ≤ 27 mg AA/kg. Liver ascorbate concentration generally increased as dietary AA supplementation level increased. Analysis by broken-line regression of WG indicated that the adequate dietary AA concentration in growing grouper was 45.3 mg AA /kg. The data also suggest that higher AA supplementation was required to improve non-specific immune responses of grouper infected with bacteria.

Table 1 Weight gain (WG), respiratory burst activity (O_2^- production ratio) of head kidney leukocyte and alternative complement (ACH50) of grouper challenge injected with *Vibrio carchariae* after fed diets containing different amounts of ascorbic acid for 8 weeks. Means in the same column followed by a different superscript letter are significantly different ($P < 0.05$)

Analyzed ascorbic acid levels (supplementation levels)	WG	O_2^- production ratio	ACH50
mg/kg	%		units/ml
□(0)	40.06 ± 8.10^a	1.32 ± 0.23^a	38.79 ± 9.56^a
3 (20)	52.37 ± 5.67^a	1.42 ± 0.22^{ab}	48.39 ± 35.11^a
14 (50)	63.83 ± 9.69^{ab}	1.48 ± 0.03^{ab}	41.35 ± 23.12^a
27 (80)	92.82 ± 16.66^{bc}	1.54 ± 0.32^{ab}	52.63 ± 6.24^a
46 (150)	121.06 ± 11.31^c	1.61 ± 0.11^{ab}	52.65 ± 2.15^a
76 (250)	124.10 ± 7.91^c	2.04 ± 0.62^{ab}	58.06 ± 7.75^a
135 (400)	129.69 ± 19.52^c	2.09 ± 0.26^{ab}	73.08 ± 12.08^{ab}
288 (800)	124.11 ± 3.16^c	2.00 ± 0.10^b	99.98 ± 13.86^b

**EFFECTS OF PROTEIN AND LIPID SOURCES ON THE GROWTH OF RED SEA BREAM
Pagrus major AND JAPANESE FLOUNDER *Paralichthys olivaceus* RECEIVING
MICROBOUND DIETS DURING LARVAL AND EARLY JUVENILE STAGE**

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The effects of dietary protein and lipid sources on growth and survival of the red sea bream *Pagrus major* (30 days old) and Japanese flounder *Paralichthys olivaceus* (15 days old) were examined using four zein-microbound diets (MBD)-containing the following nitrogen and lipid sources: MBD-1, SBP-1000 (soybean peptides with molecular weights of about 1,000) + fish oil calcium soap (FOCS); MBD-2, SBP-3000 (molecular weights of about 3,000) + FOCS; MBD-3, soybean protein isolate (SPI) + FOCS; MBD-4, SBP-1000 + stearoyl calcium lactate (SCL). Six groups of the test fish were maintained on the following diets for 30 days; group 1, live food; group 2, 1/2 live food + 1/2 MBD-1; group 3, MBD-1; group 4, MBD-2; group 5, MBD-3; group 6, MBD-4. In the red sea bream, high performance in terms of total length (TL) and body weight gain (BWG) was obtained in groups 1, 2, 3 and 4. On the other hand, the fish receiving MBD-3 (group 5) and MBD-4 (group 6) had shorter TL and lower BWG than those receiving the other diets. Regarding the Japanese flounder, high performance in terms of TL, BWG, and SR was obtained in groups 1 and 2. Group 3 receiving MBD-1 gave the BWG and SR close to those of group 2 but lower than group 1. In contrast, groups 5 and 6 receiving MBD-3 or MBD-4 alone had significantly ($P<0.05$) shorter TL and lower BWG than groups 1 and 2. The present study thus indicated that soybean peptides with molecular weights of 1,000 to 3,000 and FOCS are superior to SPI and SCL as nitrogen and lipid sources of MBD for the red sea bream and Japanese flounder at larval and early juvenile stage.

LEACHING OF SUPPLEMENTED AMINO ACIDS FROM SHRIMP DIET

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Leaching of supplemented crystalline amino acids (CAA) into water from shrimp diets and lack of knowledge of utilization of CAA by shrimp are the major obstacles for the supplementation of CAA to shrimp diet to overcome individual amino acid deficiencies in diets and to determine amino acid requirements of shrimp. Therefore, research were conducted with the aim to innovate leaching retarded CAA using several binders such as carboxymethyl cellulose (CMC), zein, *k*-carrageenan, agar, casein or gelatin to single, double or triple coating of CAA and tested on juvenile, larvae and post-larval kuruma shrimp, *Marsupenaeus japonicus* by feeding trial. Leaching loss of CAA from diets were checked 10 min, 30 min, 1 h, 6 h and 12 h after immersion into water. Coating treatments reduced the leaching of CAA during the holding period of 10, 30 min and 1 hour after immersion into water. The total amino acids leaching from the diets were lowest for the CMC bound CAA with casein-gelatin coating. Ten, 30 min and 1 h after immersion into water, more than 30%, 58% and 67% of supplemental CAA in diets with non-coated CAA were lost via leaching, respectively. With the lapse of holding time, the leaching of CAA increased in every diet; nearly 70% of CAA leached into water 12 h after immersion. The leaching rate differed with each amino acid, with different values for valine, methionine, isoleucine, leucine etc. The leaching rates of valine from the diets were highest in each sampling period compare to other indispensable amino acids. Feeding trials indicated that kuruma shrimp larvae, post-larvae and juvenile are able to utilize high amount of coated CAA. Leaching trials suggested that shrimp growth performance was improved partly due to the retardation or reduction of CAA leaching by coating them with specific binders. The innovation of leaching retarded CAA could be used to prepare environmentally sound feed for fish and crustaceans.

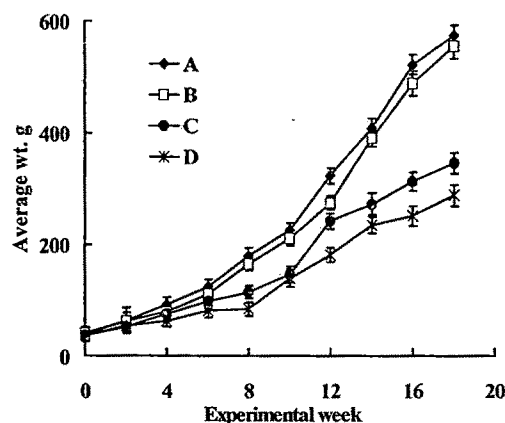
OPTIMIZED FEEDING IN THAI CATFISH *Pangasius hypophthalmus* CULTURE USING SHORT TERM FEED DEPRIVATION FOLLOWED BY SUBSEQUENT REFEEDING

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The effects of different period of starvation and subsequent refeeding on growth, survival, daily food demand, feed conversion ratio and body composition of Thai catfish, *Pangasius hypophthalmus* (initial weight 38.51 ± 1.77 g and L_T 15.71 ± 0.29 cm) were investigated. The experiment was conducted in sixteen equal sized ponds (1.5 decimal) in the field laboratory facilities of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh from June to October, 2002 for eighteen weeks. Fish maintained in four treatments were either: fed twice a day (control - A), or fed alternative day (1:1 - B), or starved 2 days followed by a spell of 2-day feeding (2:2 -C), or starved for 4 days following 4-day feeding (4:4 - D). The stocking density was 100 fingerlings per decimal. The average temperature of the pond system was 28 ± 1.54 °C. Satiation feeding was maintained for all four treatments.

The survivals were 100% in all the treatments. Thai catfish responded to a change from restricted to satiation feeding showing a higher daily feed demand compared to their counterparts raised on a liberal feeding regime. The total feed demand of fish in the control treatment was, however, much higher than the fish in other three treatments. Nonetheless, at the end of the experiment, fish that were fed to satiation on alternate day (1:1) had similar body weights to the controls, and were larger than those exposed to 2 or 4 days of feed deprivation (2:2 or 4:4). There was no significant difference in specific growth rate of fish in A and B over the experimental period. The lowest FCR was found in B at which fish were fed to satiation level in alternative day, whereas the highest FCR was found in A (control) where fish were fed to satiation twice a day. Proximate analysis of body tissue (dorsal muscle, gut and liver) was also carried out from the fish sample taken every 15-day throughout the experiment. There were no significant differences in tissue protein level in fish from different treatments. However, over the experimental period, relative gains in lipid in different tissues were highest in fish from A and lowest lipid was found in fish from D.

The study provided evidence that Thai catfish could be cultured in feeding regime with feeding in alternate day without any significant difference in fish size and final production. This way *P. hypophthalmus* farmer can save feed cost up to 30%. As the farmers are giving less feed in the system they can control water quality in a better way. In addition, this type of feeding regime also helps farmer to improve their personal time management since they do not need to attend to the fish everyday.



Individual mean weight (g) of Thai catfish in four treatments over the experimental period. Error bars are confidence limits.

DIGESTIVE ENZYME PROFILE OF CATLA *Catla catla* DURING ONTOGENIC DEVELOPMENT

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Indian major carp *Catla catla* (4 days-old) were cultured in a recirculating system for 34 days. Fish were fed with live zooplankton. The length and weight of fish showed exponential relationships with the age of fish. Amylase activity was detected from fourth day (472.2 μ g maltose/ml gut extract/h). Amylase activity was reduced to 380.4 μ g maltose/mg gut extract/h on day-10 after hatching but the activity was doubled on day-12 (770 μ g maltose/mg gut extract/h) and this showed an increasing trend thereafter. Total protease activity was minimum on day-4 (84.17 μ g tyrosine/ml gut extract) and reached maximum level on day 34 (237 μ g tyrosine/ml gut extract). Specific protease activity showed an increasing trend along with ontogenic development of fish. Trypsin activity also increased along with the ontogenic development. SDS-PAGE showed that higher molecular weight bands (ranging from 64 - 132 KDa) appeared within first 18 days, whereas lower molecular weight bands ranging from 24 - 49 KDa appeared during 20 - 34 days. Substrate SDS-PAGE showed the molecular weight of bands ranged from 33 - 121 KDa. Inhibition study with specific inhibitors showed that the enzymes belonged to the serine protease group.

EFFECT OF DIETARY PROTEIN SOURCES ON GROWTH, PROTEASE AND AMYLASE ACTIVITY OF JUVENILE *Macrobrachium rosenbergii* AT DIFFERENT GROWTH PERIODS

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A ninety-day feeding experiment was conducted on juvenile *Macrobrachium rosenbergii* on six formulated feeds at 35% protein level, to evaluate the effect of different plant and animal protein sources on the hepatopancreas protease and amylase activity. The feeds were tested for its water stability and prawns were analyzed for their proximate composition and nutritional evaluation parameters at 15, 30, 60 and 90 days. The prawns were found to utilize animal protein sources better than plant protein sources and the groups given shrimp meal and squilla meal showed the maximum total protease activity. The maximum total and specific enzyme activities were shown at 30 days and the different growth periods showed statistical significance at 5% level.

The maximum amylase enzyme activity was seen in the group fed clam meal diet at 15 days followed by those fed the silk worm pupae diet and the shrimp meal diet. The enzyme activity showed maximum levels at 30 days irrespective of the protein sources and the difference was significant at 5% level. The algae meal diet showed the least enzyme activity throughout the culture periods.

M. rosenbergii fed the squilla meal diet showed the maximum specific growth rate percentage followed by prawns fed the shrimp meal and clam meal diet. The period 15-30 days showed maximum SGR (%) which indicated that during the early stages of growth, the conversion of food material is faster than at later stages, with the animal proteins showing highest variation compared to plant proteins.

Table 1. Analysis of variance of protease specific activity at different protein sources and growth periods.

Sources	Sum of squares	Degree of freedom	Mean sum of squares	F-value
Sources	10.9815	5	2.1963	1.0011
Periods	88.0216	4	22.0054	10.0303**
Error	43.8782	20	2.1939	
Total	142.8813	29		
CD= 1.7839		CD= 1.9542		

Sources mean=13.5140 13.6940 13.3760 14.5020 14.1440 12.5860
 Periods mean= 76.2200 77.5600 96.3900 90.7500 68.1600

** significant at $p < 0.01$

IMPACT OF SUBSTRATES AND FISH STOCKING DENSITY ON THE GROWTH AND PRODUCTION OF THE INDIAN MAJOR CARP *Labeo rohita* (HAM.)

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A 70-day experiment was carried out in 9 mud-bottomed cement tanks, each temporarily partitioned into 4 equal compartments to evaluate the effect of bamboo and sugarcane bagasse substrates on the growth and production of Indian major carp, *Labeo rohita* and fish stocking density on periphyton and fish production. Bamboo poles (7.5 cm diameter) and bagasse-bundles (3.28 cm diameter) were tested in triplicate by installing at the rate of 25 and 10 per compartment respectively. The tanks were limed and fertilized with poultry manure. Fish (av. wt. 2.09 g) stocking was done at 4 densities viz. 0, 6, 12 and 18.

The final mean weights of fish were 23.22 g, 32.40 g and 32.30 g in control, bamboo and bagasse tanks. Thus, substrates affected fish growth positively (39% increase) and production increased significantly with increasing fish density. However, survival and growth of fish were independent of stocking density. Periphyton ash free dry matter did not differ significantly among substrates, but showed an increase with progress of the experiment along with that of pigment. Fish stocking density did not affect pigment content of periphyton from the two substrates. Bagasse treatment recorded the highest plankton dry weight. A reciprocal relationship was noticed between plankton dry weight and fish stocking density, indicating higher feeding on plankton at higher fish densities. Substrates reduced water temperature and dissolved oxygen. Nitrate content of water decreased over the experimental period, indicating nutrient utilization.

ABSORPTION OF AMINO ACIDS AT DIFFERENT SITES OF THE GUT IN AN INDIAN MAJOR CARP *Labeo rohita* (HAMILTON)

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The present study was designed to measure *in vivo* and *in vitro* serous transport and to quantify the rate of absorption of different essential and non-essential amino acids in the intestine of *Labeo rohita*. Experiments were conducted with eight non polar (Ala, Val, Leu, Ile, Pro, Phe, Trp, Met), nine polar (Gly, Ser, Thr, Cys, Tyr, Asn, Gln, Asp, Glu), three basic (Lys, Arg, His) and six other amino acids (2 amino n butaric acid, D L DOPA, cystine, Nor-leucine, hydroxyproline, ornithine HCl) through 'everted' and 'non-everted' gut technique. It was observed that the rate of absorption ($\mu\text{g h}^{-1} \text{sq cm}^{-1}$) was significantly higher ($P < 0.05$) in the posterior gut followed by middle and anterior gut, and recorded significant difference with the amino acids of higher (histidine – 1625.0) and lower (glycine – 884.0) molecular weight group. In general, the intestinal epithelium of lower size group of fish absorbs slightly higher amounts of amino acids as compared to larger size groups.

Out of 26 amino acids (20 natural and 6 un-natural), when considered only the essential amino acids, highest absorption was recorded with histidine (1625.0) followed by isoleucine, tryptophan, threonine, phenylalanine, valine, methionine, leucine and arginine (1027.78). This study also correlates with the electron micrograph of the intestinal lumen and confirmed that there is no absorption site at the intestinal bulb and last part of the intestinal serosa. When intact protein and crystalline amino acids included in the diet separately, a sharp increase in the plasma amino acid level was observed as compared to intact protein diet.

Although this work is a preliminary absorption study, it will help in the formulation of a balanced diet for *Labeo rohita* as well as other Indian major carps.

Table 1 Rate of absorption of EAA through serosal layer of posterior intestine.

Name of the amino acid	Rate of absorption ($\mu\text{g hour}^{-1} \text{sq cm}^{-1}$)
Valine (Val)	1425.00
Lysine (Lys)	1545.00
Isoleucine (Ile)	1545.67
Phenylalanine (Phe)	1475.67
Tryptophan (Trp)	1500.00
Methionine (Met)	1416.67
Threonine (Thr)	1500.00
Arginine (Arg)	1027.78
Histidine (His)	1625.00
Ornithine HCl	1441.67

EVALUATION OF MIXED FEEDING SCHEDULES WITH VARYING DIETARY PROTEIN CONTENT ON THE GROWTH PERFORMANCE AND REDUCTION OF COST OF PRODUCTION FOR PANGAS WITH SILVER CARP

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An on-station pond trial was conducted for 6 months to investigate the suitability of mixed feeding schedules with varying protein levels on the growth of pangas, *Pangasius hypophthalmus*, and silver carp, *Hypophthalmichthys molitrix*, with a view to minimizing the cost of production of pangas. The five different feeding regimes employed were: high protein diet (30% protein, H), low protein diet (15% protein, L), one day low protein diet followed by one day high protein diet (1L/1H), 7 days low protein diet followed by 7 days high protein diet (7L/7H) and 14 days low protein diet followed by 14 days high protein diet (14L/14H). Ten experimental ponds each of 80 m² size were divided into 5 treatment groups each with two replicates. Fishes were stocked at the ratio of 80:20 (pangas:silver carp) at the total rate of 25000/ha. Feeding rate was calculated on the basis of pangas weight only and fish were fed at the rate of 15, 10, 8 or 5% of their body weight for the 1st, 2nd, 3rd, and subsequent months, respectively. The range of water quality parameters during the study period were: temperature 22 to 32.8°C, dissolved oxygen 4.30 to 7.44 mg/l, pH 6.85 to 7.50 and transparency 21.9 to 32.2 cm.

The result of the study showed that significantly ($P < 0.05$) higher weight gain of fishes was achieved in feeding schedules of H and 1L/1H and lowest with 14L/14H. The FCR values ranged between 2.06 to 2.51 with H and 1L/1H showing significantly the lowest i.e. the best FCR. The FCR values were very low when weight of silver carp was considered which ranged between 1.58 to 1.68. The survival (%) of pangas were lower (78.1 to 82.5%) compared to that of silver carp (90-95%). Total production of pangas ranged between 3594 to 7095 kg/ha and silver carp ranged between 1777 to 2220 kg/ha. A simple economic analysis showed that fish in 1L/1H fed alternately with low and high protein diets resulted in the highest net profit. The results of the study demonstrated that feeding of fish continuously with high protein diet is not economical. For profitable pangas farming in Bangladesh, farmers can adopt a mixed feeding schedule of alternated low and high protein diet instead of continuously feeding a high protein diet.

Table 1 Final weight (g), production (kg/ha) and net profit (Tk/ha) of pangas and silver carp maintained under different feeding schedules for 6 months

Parameters	Feeding schedules				
	H	L	1L/1H	7L/7H	14L/14H
Mean initial weight (pangas)	2.5	2.5	2.5	2.5	2.5
Mean initial weight (silver carp)	25.0	25.0	25.0	25.0	25.0
Mean final weight (pangas)	415.0 ^a	230.0 ^d	430.0 ^a	385.0 ^b	275.0 ^c
Mean final weight (silver carp)	465.5 ^b	395.0 ^e	480.2 ^a	445.2 ^c	415.1 ^d
Production (pangas)	6795 ^b	3594 ^e	7095 ^a	6072 ^c	4306 ^d
Production (silver carp)	2211 ^a	1777 ^b	2220 ^a	2114 ^a	1864 ^b
Total production (kg/ha)	9006 ^b	5371 ^e	9315 ^a	8186 ^c	6170 ^d
Net profit (Tk/ha)	75097	58806	136441	105318	52687

1US dollar = Taka 58

Figures in the same row having the same superscripts are not significantly different ($P > 0.05$).

GROWTH PERFORMANCE, FEED UTILIZATION EFFICIENCY AND DIGESTIVE ENZYME ACTIVITY IN ROHU *Labeo rohita* FINGERLINGS FED YEAST EXTRACT POWDER (YEP) SUPPLEMENTED DIETS

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Yeast extract powder (YEP) consists of protein and other intracellular constituents extracted by the process of autolysis. It is a rich source of B vitamins and is used to supply these factors in bacterial culture. The objective of this study was to evaluate the effects of YEP supplemented diets on growth performance and digestive enzyme activity (both intestinal and gut bacterial) in rohu, *Labeo rohita*, fingerlings. Rohu fingerlings (1.76 ± 0.15 g) were fed fish meal based 35% protein diets supplemented with 0.1% (diet D1), 0.2% (D2), 0.3% (D3), 0.4% (D4) and 0.5% (D5) YEP for 75 days twice daily (2% body weight) in triplicate treatments. The control diet (RD) was not supplemented with YEP.

Rohu fingerlings fed YEP supplemented diets exhibited better growth in comparison to the RD up to 0.2% level. YEP at 0.1% level resulted in the best performance of the fish in terms of % weight gain, SGR, FCR and PER followed by 0.2% level. Apparent protein digestibility values showed a decreasing trend with increasing level of YEP inclusion. YEP led to higher deposition of protein and depletion of lipid in the carcass. Diet D1 resulted in maximum deposition of protein in the carcass followed by D2. The intestinal α -amylase activity was maximum with rohu fed D2, while the protease activity was maximum with D1. The enzyme producing capacity of gut bacterial flora in *in vitro* culture followed the same trend (Table 1). The results indicated a positive effect of oral administration of YEP on rohu fingerlings. The better growth performance and protein digestibility in rohu fingerlings fed YEP supplemented diets up to a level of 0.2% might be due to the production of extracellular enzymes by gut microflora which helped in better utilization of feed. Further studies are required to characterize (physiological and biochemical) and identify these microorganisms prior to full scale application in commercial aquaculture.

Table 1 Activities of digestive enzymes in rohu fingerlings fed experimental diets for 75 days. The results are mean $\pm$ SD of 3 determinations. Means in the same column with same superscript are not significantly different ($P>0.05$).

Diets	Protease activity		α -Amylase activity	
	Intestinal ^a (μ g glycine liberated h^{-1} mg protein ⁻¹)	Bacterial ^c (diameter of halo, mm)	Intestinal ^b (mg maltose liberated h^{-1} mg protein ⁻¹)	Bacterial ^c (diameter of halo, mm)
RD	22.13 $\pm$ 0.92 ^c	8.5 $\pm$ 0.4 ^c	10.15 $\pm$ 0.34 ^d	ND
D1	28.15 $\pm$ 1.28 ^a	16.2 $\pm$ 0.6 ^a	13.19 $\pm$ 0.54 ^{ab}	4.5 $\pm$ 0.2 ^c
D2	27.02 $\pm$ 1.29 ^a	13.5 $\pm$ 0.5 ^b	13.53 $\pm$ 0.44 ^a	10.2 $\pm$ 0.5 ^a
D3	23.89 $\pm$ 1.05 ^{bc}	9.0 $\pm$ 0.4 ^c	10.82 $\pm$ 0.49 ^d	10.2 $\pm$ 0.5 ^a
D4	24.27 $\pm$ 1.18 ^b	8.5 $\pm$ 0.3 ^c	12.56 $\pm$ 0.56 ^{bc}	8.0 $\pm$ 0.3 ^b
D5	22.03 $\pm$ 1.06 ^c	6.2 $\pm$ 0.2 ^d	12.19 $\pm$ 0.58 ^c	7.5 $\pm$ 0.2 ^b

^aInitial value : 14.69 $\pm$ 0.58 ; ^bInitial value : 7.14 $\pm$ 0.29

^cWith pure culture of intestinal isolates. ND : Not detected

POPULATION DYNAMICS OF A COMMON BRACKISH WATER ZOOPLANKTON SPECIES ENRICHED WITH COMMERCIAL PROBIOTICS, INERT FEED AND BAKER'S YEAST

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Zooplankton being an important food organism for shrimp, its population need to be well maintained in a pond system. The traditional method of pond fertilization to induce primary productivity hence to stimulate zooplankton production, however, is questionable of late, following an excessive presence of nuisance algae in the culture environment. Giving due consideration to the baker's yeast, which has a proven track record for producing the much needed zooplankton in the culture system, this study looked into the possible use and the efficacy of other products such as the inert feed in the form of prepared detritus and commercial probiotic. Hence, an initial inoculation of zooplankton was introduced, consisting of a combination of adult and nauplii stages of copepod *Oithona* sp, L-type rotifer *Brachionus plicatilis* and dwarf rotifer *Collurella adriatica* at a density of 0.40, 0.60, 0.34 and 0.24 individual ml⁻¹ respectively into each of the twelve fiber glass tanks filled with 200 l of 5µ filtered sea water at salinity of 20 ppt (28.0 ± 1.5 °C, pH 8.1 ± 0.2). In triplicates, the cultures were enriched twice daily with diets consisting of baker's yeast, prepared detritus, commercial probiotic bacteria type 'A' and type 'B' product. Practiced in static water culture system, water quality parameters such as water temperature and pH were recorded twice daily while ammonia nitrogen was recorded once every three days. All the different zooplankton species inoculated in the system indicated a steady propagation but differed significantly in overall growth dynamic pattern. For a speedy propagation of the organisms, baker's yeast was the best enrichment product but it could not ensure sustainable zooplankton production. Next to it is the inert feed in the form of detritus however, it performed better ($P < 0.05$) in terms of sustainable zooplankton production. The commercial probiotic bacteria products were slower in stimulating zooplankton production. However, the probiotics induced the best zooplankton growth dynamic and the most sustainable propagation. After about two weeks of enrichment, the zooplankton population in the culture systems enriched with both the commercial bacteria probiotic products and the detritus prepared from inert feed found to produce three times more zooplankton population if, compared to the culture system enriched with baker's yeast. In all the different treatments, the organism that multiplied the most was the rotifer, *B. plicatilis*. Its dominance, somehow, managed to suppress the other zooplanktons, which are equally important to the shrimp in the culture system, within a short period of 5 to 7 days of the propagation period. Practically, it was found that the culture system, enriched with the different products did not deteriorate water quality.

**DEVELOPMENT OF A SUITABLE DIET USING LOCALLY AVAILABLE FEED
INGREDIENTS FOR MONOCULTURE OF GIANT FRESHWATER PRAWN
Macrobrachium rosenbergii IN PONDS**

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An experiment was conducted for three months in 12 experimental ponds of each 30m² size. Three experimental diets containing 30% protein were formulated using fish meal, meat and bone meal, mustard oilcake, sesame meal and rice bran. A special shrimp feed (starter -II) from Saudi-Bangla Fish Feed Ltd. was used as the control (diet 4). Juveniles of *Macrobrachium rosenbergii* (2.90±0.81g) were stocked at the rate of 4/m². They were fed twice daily at the rate of 10% of their body weight at the beginning, which was gradually reduced to 5% for the last month. The ponds were provided with aeration during the night using air pumps. The results of the study showed that there was no significant ($P>0.05$) difference between the weight gains of prawns fed diets 1 and diet 4 (control), but was significantly ($P<0.05$) higher than other dietary groups. The FCR values of diets ranged between 2.61 to 3.36 with diet 1 and 4 showing significantly ($P<0.05$) lower values. The survival ranged between 68 to 78% with prawns fed diets 1 and 4 showing significantly higher survival. The production ranged between 921 to 1428 kg/ha/90days and diet 1 resulted in significantly ($P<0.05$) highest production. A simple economic analysis showed that diet 1 generated maximum net profit of Tk. 161,980/ha/90days. The results of the study showed that a diet containing 20% fish meal, 10% meat and bone meal, 15% mustard oilcake, 15% sesame meal, 35% rice bran, 4% molasses and 1% mineral-vitamin premixes could be recommended for monoculture of *M. rosenbergii* in ponds which is cheaper than commercial prawn diets available in the market.

Table 1. Growth and feed utilization in *Macrobrachium rosenbergii* fed with experimental diets for 3 months.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Mean initial weight(g)	2.90 ^a	2.90 ^a	2.90 ^a	2.90 ^a
Mean final weight(g)	46.32 ^a	39.30 ^b	34.36 ^b	40.43 ^{ab}
Weight gain(g)	43.42 ^a	36.40 ^b	31.46 ^b	37.53 ^{ab}
SGR(%/day)	3.12 ^a	2.90 ^{bc}	2.74 ^c	2.92 ^b
FCR	2.61 ^a	3.16 ^b	3.36 ^b	2.80 ^a
PER	1.24 ^a	1.03 ^b	0.99 ^b	1.16 ^a
Survival(%)	78 ^a	72 ^{ab}	68 ^b	75 ^{ab}
Total production (Kg/ha/90 days)	1427.6 ^a	1118.9 ^c	921.3 ^d	1197.9 ^b
Net profit (Tk./ha/90days)	161,980	76,618	29,702	39,683

1 US dollar = Taka 58

¹Figures in the same row with same superscripts have no significant variation ($P>0.05$)

INTERACTIVE EFFECTS OF DIETARY PROTEIN AND CARBOHYDRATE LEVELS ON GROWTH, NUTRIENT UTILIZATION AND BODY COMPOSITION IN ROHU *Labeo rohita* FINGERLINGS

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A 60-day feeding trial was conducted on replicate groups of rohu, *Labeo rohita*, fingerlings (5.48 ± 0.02 g) to study the effect of increasing dietary levels of carbohydrate at varying dietary protein levels on growth, nutrient utilization efficiencies and protein sparing effect of carbohydrate. A restricted ration @ 2% BW d⁻¹ once daily was employed using twelve purified diets with casein, gelatin and dextrin prepared to achieve 25, 35, 40 and 45 % protein and 15, 25 and 35 % carbohydrate at each specified protein level. The gross energy level was 336.00 ± 15.47 , 377.00 ± 17.13 and 417.00 ± 15.77 kcal/100g corresponding to 15, 25, and 35% carbohydrate diets, respectively, while the P/E ratio corresponding to dietary protein levels ranged between 63.42 and 78.81 mg/kcal (25% protein), 85.44 and 105.15 mg/kcal (35 % protein), 98.74 and 126.77 mg/kcal (40 % protein) and 103.75 and 127.01 mg/kcal (45% protein).

The best performance of fish in terms of weight gain %, SGR, DGI, ADG, FCR and PER was recorded in diet P40/C35 (40% protein and 35 % carbohydrate). Although the overall growth was lower with low protein diets (P25/C15 to P35/C25), the increased supply of energy from carbohydrate at each protein level induced better growth rates and protein efficiency ratio. Protein sparing effect was at the threshold when dietary energy level was augmented by inclusion of carbohydrate at 40% protein level. The interaction of dietary protein (X_1) and carbohydrate (X_2) level on percentage weight gain (Y) was established from the multiple regression analysis which explained 91.95% variation due to protein and carbohydrate in the diet :

$$Y = -116.245 + 4.216X_1 + 2.179X_2 \quad (r^2 = 0.9195)$$

The dietary protein rather than dietary carbohydrate was the major determinant of growth over the range of protein and energy levels tested. But within the same protein level, lower P/E ratio (higher carbohydrate %) yielded better growth. The amount of feed and protein remaining same, FCR was closely identical to the trends for weight gain indicating that the fish was able to use carbohydrate as an energy source. The interactive effect of dietary protein and carbohydrate levels on PER and FCR was low but significant ($P < 0.05$) as indicated from regression equations:

$$Y_{PER} = 0.2047 + 0.0144 X_{1(\text{Protein})} + 0.0305 X_{2(\text{Carbohydrate})} \quad (r^2 = 0.535)$$

$$Y_{FCR} = 23.7353 - 0.1081 X_{1(\text{Protein})} - 0.8965 X_{2(\text{Carbohydrate})} \quad (r^2 = -0.6699)$$

Protein consumption per kilogram bodyweight gain at all protein levels marked significantly lower values as the carbohydrate level rose from 15 to 35%, indicative of protein sparing action of carbohydrate on fish. The apparent digestibility of protein and lipid were not altered by the dietary treatments. At all of the protein levels tested, dietary carbohydrate did not influence significant changes in the body composition.

The study established the possibility of incorporating up to 35% carbohydrate in compound diets with 40% dietary protein content (gross energy : 413 kcal/100g; P/E ratio: 98 mg/kcal) to support optimal growth rate in rohu fingerlings. The protein sparing effect of dietary carbohydrate was confirmed from the highly significant positive effect on growth and nutrient retention efficiencies.

USE OF L-ASCORBYL-2-MONOPHOSPHATE-NA/CA AS A VITAMIN C SOURCE FOR JUVENILE KURUMA SHRIMP *Marsupenaeus japonicus* (BATE)

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A feeding trial was conducted to determine the vitamin C (Ascorbic acid, AsA) effect on growth and stress resistance of kuruma shrimp juvenile, *Marsupenaeus japonicus* using L-ascorbyl-2-monophosphate-Na/Ca (AMP-Na/Ca) as a vitamin C source. Six different levels of AMP-Na/Ca with AsA equivalent levels of 0, 48, 92, 165, 367 and 745 mg kg⁻¹ diet were supplemented in semi-purified diets and fed to juvenile shrimp, *M. japonicus* (mean weight 0.31 ± 0.04) for 60 days. Each treatment was set up with four replications. After the feeding trial, shrimp were subjected to osmotic stress to evaluate the effect of AsA on stress resistance. Results indicated that the highest weight gain (WG, %) and specific growth rate (SGR, %) was found in 48 mg AsA kg⁻¹ diet. However, there was no significant difference in feed efficiency ratio (FER) (P>0.05). Poor survival was found in the shrimp fed the AsA free diet after 40 days. Shrimp survival was significantly reduced at the end of the trial by feeding a vitamin C-deficient diet (P<0.01). Tolerance against osmotic stress was significantly higher in diets containing 367 and 745 mg AsA kg⁻¹ compared to others. Generally, AsA contents in the hepatopancreas of shrimp increased with dietary AsA levels. The hepatopancreatic AsA of shrimp fed an AsA free diet showed a significantly lower value than those fed the diets containing 367 and 745 mg AsA kg⁻¹, but the values for the latter two groups were not significantly different.

IMPACT OF FEEDING STRATEGIES ON THE GROWTH AND BODY COMPOSITION OF STRIPED CATFISH *Pangasius hypophthalmus*

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Striped catfish *Pangasius hypophthalmus* is one of the most economically important freshwater fish in Southeast Asian countries such as Thailand, Vietnam, and Cambodia. Its high growth rate and adaptability to various food and culture conditions make it an ideal candidate for aquaculture. But the poor carcass quality resulting from high fat content and off-flavor have reduced its farm-gate price. It was suspected that the use of inappropriate feeds and feed management might have exacerbated the problem of fat deposition in fish, and this may be linked to the occurrence of off-flavor. An on-farm survey was conducted to evaluate the effect of different feeding regimes and culture management practices of commercial farms on the body composition of striped catfish. Fifteen farms in Central of Thailand with different feeding systems and feeding regimes were randomly selected for this study. The general information on culture practices, farm economics were obtained by interviewing farmers using a prepared questionnaire. Fish of marketable size (1-1.5 kg) were collected from each farm for carcass proximate composition analysis.

There were significant differences in proximate body composition of fish among the 15 surveyed farms in this study. The moisture content varied widely in sampled fish (57-73%). The highest lipid content (59%, dry weight basis) was observed in fish fed leftover bread and the lowest lipid content (22%) was found in fish raised with integration of chicken. There were inverse relationships between the lipid and protein contents or lipid and moisture contents of the fish. Body protein content of fish also varied greatly (35-63%) among the fifteen farms. The carcass ash was between 8.7-15.2%. The average body lipid of striped catfish from commercial farms was 39.9% and the fish from wasted food-fed farm contained 50% of body lipid. The body lipid of fish grown in integrated farms had lower fat content (26.7%) and fish cultured in integrated farms but received additional feed had intermediate lipid levels (37.7%).

Since the result of the survey showed that on-farm feeding regimes affected the body composition of striped catfish, a feeding trial was conducted to investigate whether it is possible to control body lipid content of fish by manipulating dietary composition. The feeding trial used nine practical diets containing three levels of protein i.e. 20, 25, 30%. The P:E ratio of each protein level were manipulated by varying dietary fiber 7, 13 and 23%, respectively. The corresponding lipid levels of the diets at each protein level were 12, 7 and 2%, respectively. The juvenile fish with initial average weight of 157 g were stocked in 27 cages installed in 3 earthen ponds. Fish were fed to satiation twice a day with the experimental diet for 3 months. At the end of experiment, fish were weighed and analyzed for proximate body composition. There was no significant difference in weight gain, FCR, PER and ANPU ($P < 0.05$) among treatments. Body protein content varied from 40.6% to 63.5%. The highest percentage of body protein was obtained with fish fed 20% protein diet that contained 23% and 2% of fiber and lipid respectively. The lowest percentage of body protein was also found in fish fed 20% protein but with 7% fiber and 12% lipid (the lowest P:E ratio). At each protein level, percentage of body protein decreased with a decrease in P:E ratio. Body lipid contents follow the opposite trend of protein percentage. Percentage of body lipid varied from 27 to 48% and the highest body lipid was observed in fish fed with the lowest P:E ratio diet containing 20% protein. Manipulation of P:E ratio by decreasing dietary lipid and increasing dietary fiber produced fish with lower of body lipid.

EFFECTS OF DIETARY SUBSTITUTION OF TUNA MUSCLE BY-PRODUCT POWDER FOR FISH MEAL ON GROWTH, BODY COMPOSITION AND PHOSPHORUS LOADING IN JUVENILE JAPANESE FLOUNDER *Paralichthys olivaceus*

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Tuna muscle by-product powder (TMP) was evaluated as fish meal substitute in Japanese flounder (*Paralichthys olivaceus*) diet. Control diet containing 45 % protein was formulated using fish meal as the major protein source. TMP was used to replace 25 %, 50 %, 75 % or 100 % fish meal, and effects on weight gain, feed intake, and phosphorus retention were determined in a 40-day growth trial. The feed intake and weight gain of fish fed the control diet, 25 % or 50 % replacement diets were not significantly different; however, they were inversely related to inclusion levels of TMP up to 50 % replacement. Phosphorus (P) concentration of the experimental diets decreased with increasing dietary TMP substitution. Whole body P concentration was not significantly affected by dietary treatment, and P loading markedly decreased with increasing TMP concentration in diets. These results suggest that TMP may be promising protein source for inclusion to reduce discharge of phosphorus into the water, and can replace 50 % of fish-meal protein with no adverse effects on weight gain and survival of *P. olivaceus* juveniles. Further researches concerning the relative palatability of TMP should be conducted.

**EFFECT OF DIETARY PROTEIN-TO-LIPID RATIO ON GROWTH AND BODY
COMPOSITION OF BAGRID CATFISH *Mystus nemurus***

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A 16-week feeding trial was carried out to evaluate the utilization of lipid as dietary energy source for juvenile bagrid catfish (*Mystus nemurus*). Eight isonitrogenous and isocaloric semi-purified diets were fed to triplicate groups of *M. nemurus* (initial mean weight of 3.88 ± 0.14 g). A total of eight diets were prepared with two protein levels (35% or 40%) were incorporated at four different lipid levels (5, 10, 15, 20%). The diets were designated P₃₅L₅, P₃₅L₁₀, P₃₅L₁₅, P₃₅L₂₀, P₄₀L₅, P₄₀L₁₀, P₄₀L₁₅, P₄₀L₂₀, respectively. Overall best significant growth performance was obtained for fish fed the P₃₅L₂₀ diet with an average relative growth rate (RGR) of 116.92%, followed by P₃₅L₁₅ (103.80%) and P₄₀L₂₀ (87.1%). Survivor was high, there was no significant differences ($P > 0.05$) were found among treatments. Nutritive parameters such as feed intake, food conversion ratio (FCR) and protein efficiency ratio (PER) were affected by diets. The significant differences were recorded in percentage of body indices such as intra-peritoneal fat, hepatosomatic index and body composition of bagrid catfish at different dietary protein and lipid levels ($P < 0.05$). Carcass lipid content were inversely related to moisture content. These study indicates that the protein sparing capabilities of juvenile bagrid catfish and the results suggest that diet containing 35% protein and 15% lipid with P:E ratio of 20.91 mg protein kJ-1 is optimal for the candidate species.

CHEMICAL POTENTIAL OF LABLAB

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Chemical characterization of *lablab* (benthic complex) was done to determine the potential metabolic compounds and nutrients present. The *lablab* collected from Leganes, Iloilo, Philippines was predominantly composed of microalgae; *Chaetoceros spp.*, *Pleurosigma spp.*, *Oscillatoria spp.*, and unidentified filamentous algae, and the rotifers, *Brachionus spp.* Qualitative tests and infrared (IR) spectroscopy confirmed and established the presence of terpenes, saponins, cardiac glycosides, phenolics, flavonoids, fatty acids and amino acids in the *lablab* extracts.

Bioassay of the different extracts proved that the dichloromethane (DCM) fraction has significant antibacterial activity against *V. harveyi* apparently due to phenolic and flavonoid compounds detected. Phenolics and flavonoids are purportedly significant antimicrobial compounds. Nutrient analysis of the *lablab* samples showed that $5.81 \pm 0.28\%$ crude protein with all the essential amino acids, $6.11 \pm 0.11\%$ crude lipid to include both saturated and unsaturated fatty acids, $0.12 \pm 0.02\%$ crude fiber, and nitrogen-free extract of 8.91% were present. The $79.05 \pm 0.02\%$ mineral content was significantly rich in essential elements such as calcium (1155 mg/L), phosphorus (2037 mg/L), iodine (2795 mg/L), iron (36390 mg/L), and zinc (67 mg/L).

The presence of these compounds in *lablab* made them significantly valuable to aquaculture as an excellent live feed and a potential source of bioactive compounds with promising antibacterial property.

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EFFECTS OF DIETARY CARBOHYDRATE TO LIPID RATIO WITH TWO LEVELS OF PROTEIN ON THE GROWTH PERFORMANCE, BODY COMPOSITION AND HEPATOSOMATIC INDEX OF RAINBOW TROUT *Oncorhynchus mykiss*

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Effects of carbohydrate to lipid ratio at two different protein levels on the rainbow trout (*Oncorhynchus mykiss*) was investigated in this feeding trial. In this experiment, fish- with average initial weight of 60 ± 0.55 g were used and the feeding trial conducted for 60 days in 500 liter tanks. A 2x2 factorial experiment with 2 levels of protein (40 and 45%) and 4 ratios of carbohydrate to lipid (0.5 , 1 , 1.5 and 2) with 4000 Kcal/kg constant energy were formulated and prepared. The study was conducted with 8 treatments and triplicate random groups of 20 fish per tank. Fish biomass were determined at fortnightly intervals and daily feed levels adjusted accordingly. Results showed that with increase in dietary protein levels, growth performances did not improve significantly ($P \geq 0.05$) . The results of study also indicated that at constant protein level, weight gain, SGR, FCR, PER and price index improved with decrease in carbohydrate to lipid ratio and fish fed a carbohydrate to lipid ratio of 0.5 had better growth performance than fish fed other ratios. Furthermore, results showed that fish fed less protein and carbohydrate / lipid ratio had the best condition without any negative performance on the liver. According to results obtained, it could be suggested that for this size of rainbow trout, a dietary protein level of 40% and carbohydrate / lipid ratio of 0.5 can be used

**EFFECT OF TEMPERATURE, SALINITY AND STOCKING DENSITY ON DEVELOPMENT
OF MASS CULTURE OF *Isochrysis galbana* AND *Nannochloropsis oculata* FOR
BREEDING**

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In 2002 – 2004, Research Institute for Aquaculture No. 1 (RIA1) in Vietnam have produced 450.000 seeds of *Brown spotted - grouper* *Epinephelus coioides* for 60 days (4 - 5cm length) on an experimental scale. In order to raise mass culture of two micro-algal species *Isochrysis galbana* and *Nannochloropsis oculata* the effect of temperature, salinity and stocking density on production was studied. Salinity (25‰), temperature 25 °C and stocking density 2,106 and 5,106 with *I.galbana* and *N.ocullata* respectively gave the best response for algal mass culture. Harvest of *I.galbana* and *N.ocullata* at average density of 26.27 - 28.13x10⁶cell.ml⁻¹ and 22.57x10⁶ cell.ml⁻¹ is effective and economical.

USE OF LOW FISHMEAL FEEDS IN AQUACULTURE OF THE COBIA *Rachycentron canadum*

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One of the most challenging tasks facing the sustainable development of aquaculture is the strategy to produce feeds that are composed of minimum level of fishmeal and fish oil for the mostly carnivorous fishes under cultivation. A carnivorous pelagic fish, the cobia (*Rachycentron canadum*) grows rapidly and is becoming a key candidate species for sea cage aquaculture in tropical coasts. In an effort to minimize effectively the use of fish meal in diets of the cobia, a series of experiments have been conducted to identify the proper alternative protein sources, the strategy of their effective incorporations, and nutritional problems associated with high level supplement of the alternative proteins. The results have so far indicated that the cobia can effectively use non-fishmeal protein ingredients. Up to 40% of the fish protein in the cobia feed could be replaced by soybean meal without compromising growth. Biological or chemical treatment of soybean that altered its flavor and chemical compositions changed the nutritional responses of the cobia. Lupin meal, blood meal, and canola meal also are effective alternative protein sources. Use of supplemental crystalline amino acids or their modified products in the low fishmeal feeds has been found ineffective in supporting growth of the juvenile cobia. In addition to problems relating to protein quality degradation during fishmeal substitution, the presence of phytate in the plant proteins and its anti-nutritional role in the cobia were studied. The results have indicated that the cobia could effectively utilize the plant proteins commonly used for aquaculture feed production. Methods designed to enhance the utilization have achieved some benefits. Further studies are needed to fine tune and thus improve the strategy to optimize the incorporation of alternative protein sources in the feed of the cobia.

PARTIAL REPLACEMENT OF FISHMEAL BY SOYBEAN MEAL IN THE DIET OF ROHU *Labeo rohita* (HAMILTON) FRY

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A laboratory feeding trial has been conducted for 8 weeks with *Labeo rohita* fry in 12 aquaria in static indoor fish rearing system of the Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh, to evaluate the effect of protein of soybean meal (SBM) and fishmeal on the growth and survival of Indian major carp, *L. rohita* fry. In this trial, 3 diets were used as Diet 2 (75% fishmeal protein and 25% soybean meal protein), Diet 3 (50% fishmeal protein and 50% soybean meal protein), Diet 4 (25% fishmeal protein and 75% soybean meal protein) without supplementation of amino acid. The fry were fed on a pelleted diet containing 30% crude protein having fish meal and soybean meal as major protein source except control (Diet 1) where only fishmeal was used. Performances of the formulated diets were compared with the control diet (Diet 1). Feeding with 25% soybean meal plus 75% fish meal (Diet 2) based diet resulted in significantly ($P<0.05$) better performance of *L. rohita* in terms of live weight gain (%), specific growth rate (SGR % day⁻¹) and feed conversion ratio (FCR) as compared to those fed continuously with 100% fish meal and other diets (Diets 3 and 4). Higher protein deposition in carcass and higher protein efficiency ratio (PER) were also found in fish fed with Diet 2. There was no significant ($P<0.05$) difference in fish growth and feed efficiency of fish fed diets 1 and diet 3. The highest FCR was obtained in the fish reared on Diet 4, which was significantly ($P<0.05$) different from all other diets. However, the feeding performances of Diet 4 was significantly ($P<0.05$) lower than those of other dietary groups. The findings of the present study suggest that *L. rohita* fry can be effectively reared with Diet 2 where 25% fishmeal was replaced by soybean meal. The growth response and feed utilization of fish fed Diet 3 was also comparable with that of control. Therefore, considering the price and scarcity of fishmeal, up to 50% soybean meal can be used for the diet of *L. rohita* without supplementation of amino acids.

Table 1 Performance of *Labeo rohita* fry fed on experimental diets for 8 weeks.

Parameters	Diet-1	Diet-2	Diet-3	Diet-4
Average live weight gain (%)	205.80 ^b	245.40 ^a	200.00 ^b	134.90 ^c
Final body weight (g)*	2.63 ^b	2.97 ^a	2.58 ^b	2.02 ^c
SGR (%/day)	2.95 ^b	3.52 ^a	2.87 ^b	1.93 ^c
PER	1.40 ^b	1.65 ^a	1.45 ^b	1.06 ^c
FCR	2.29 ^b	2.02 ^c	2.30 ^b	3.14 ^a
ANPU(%)	21.03 ^b	24.49 ^a	21.13 ^b	16.10 ^c
Survival rate (%)	100	100	100	100

*Initial body weight 0.86g

Values having the same superscripts in the same row are not significantly different ($P<0.05$).

POTENTIAL OF SUBSTRATE BASED MICROBIAL BIOFILM FOR NURSERY REARING OF MRIGAL *Cirrhinus mrigala* AND FRESHWATER PRAWN *Macrobrachium rosenbergii* IN CEMENT TUBS

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Two experiments were conducted separately in 1m³ cement tubs to study the effect of substrate on water quality, biofilm and plankton production and growth and survival of *Cirrhinus mrigala* and *Macrobrachium rosenbergii*, for a rearing period of 28 days each. In the first experiment three tubs each received cow dung @ 1 kg/tub (T₁), cow dung @ 1kg/tub + feed (fish meal based) @ 10% of body weight/day (T₂) and cow dung @ 1kg + substrate (paddy straw) @ 200 g/tub (T₃). All the tubs were stocked with 10 fry of *C. mrigala* (0.09 g) 7 days after the addition of manure and substrate. In the second experiment three tubs each received substrate, sugarcane bagasse (T₁) and paddy straw (T₂) and three tubs without substrate served as control (T₃). All the tubs were manured with poultry droppings @ 2 kg /tub and stocked with 15 post larvae/tub of *M. rosenbergii* (0.01g) 7 days after manure and substrate addition. Important water quality parameters were analyzed initially and after the addition of manure and substrate at weekly intervals in both the experiments.

In the first experiment the water temperature, pH, dissolved oxygen, alkalinity and carbon dioxide recorded ranged from 24.6 to 27.8 °C, 7.01 to 7.99, 3.38 to 6.75 mg/l, 30 to 68 mg/l and 0 to 15.8 mg/l respectively. Tubs without substrate recorded higher average ammonia of 7.99 (T₁) and 9.15 µg-at./l (T₂) than tubs added substrate (6.55 µg-at./l). Total plate count (TPC) of bacteria (CFU x 10⁴/ml) in water did not vary much between the treatments and the values were 5.13, 5.49 and 5.85 in T₁, T₂ and T₃ respectively. TPC on substrate (CFU x 10⁶/g) was higher than in water and the average value was 1.34. Significantly higher phytoplankton and zooplankton were recorded in T₂ (P< 0.05) compared to T₁ and T₃ whereas higher phytoplankton was recorded in T₃ compared to T₁. Phytoplankton and zooplankton recorded on substrate were 203 and 70.67 no./cm². Growth of *C. mrigala* was significantly higher (P< 0.05) in substrate added tubs; the average final weight recorded being 3.37, 5.01 and 6.93 g in T₁, T₂ and T₃ respectively.

In the second experiment the mean water temperature, pH, dissolved oxygen, alkalinity and carbon dioxide recorded ranged from 25.1 to 28.1°C, 7.23 to 7.99, 3 to 6.38 mg/l, 40 to 78 mg/l and 0 to 14 mg/l respectively. Total ammonia did not differ significantly between the treatments T₁ (5.19) and T₂ (5.32) but both differed significantly with control T₀ (7.16). TPC of bacteria in water (CFU x 10⁴/ml) did not vary much between the treatments with the mean values recorded being 6.07, 6.8 and 6.46 in T₀, T₁ and T₂ respectively. Bacterial counts on bagasse (CFU x 10⁶/g) was higher (2.06) than paddy straw (1.54). Higher density of phytoplankton and zooplankton were recorded on bagasse than paddy straw. Average final weight and survival of *M. rosenbergii* recorded were 0.83g and 62.22%, 1.28g and 86.67%, and 0.99 g and 80% respectively in T₀, T₁ and T₂.

FEED ATTRACTANTS FOR THE FRESHWATER PRAWN *Macrobrachium rosenbergii*

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Feed attractants play a significant role in the economic success of aquaculture as they optimize feed conversion rates by maximizing consumption and reducing feed wastes so as to lower production cost. Use of attractants in prawn feed is reported to reduce feed waste and improve the feed efficiency. Considering the importance of attractants, a study was carried out to evaluate several natural materials as attractants in the feed of freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879). Different feeds were prepared by varying percentage incorporation of attractants. The attractants used were fried coconut cakes, peenari, cuttle fish liver, squid meal and prawn head.

The composition of different feeds is given in Table 1. Feed 1 does not contain attractants and served as control. Proximate composition of different feeds were analyzed and reported in Table 2. Proximate composition does not significantly vary for the different feeds. Highest water stability value was recorded for Feed 6 containing prawn head as attractant. Highest values of SGR, FCE and PER were recorded for Feed 5 in which squid meal was used as attractant. From the results, it is inferred that a feed containing squid meal as attractant was the best and it provides greater feed utilization and growth in *Macrobrachium rosenbergii*.

Table 1 Feed Composition (%)

Feeds	Clam	GOC	Wheat bran	Tapioca	Attractants*	Total
Feed 1	40	25	25	10	0	100
Feed 2	40	25	24	10	1	100
Feed 3	40	25	24	10	1	100
Feed 4	40	25	24	10	1	100
Feed 5	40	25	24	10	1	100
Feed 6	40	25	24	10	1	100

*Attractants used in various feeds were Feed 1-control, Feed 2 -Fried coconut cake, Feed 3 -Peenari ,Feed 4- Cuttle fish liver, Feed 5- Squid meal, Feed 6 -Prawn head

Table 2 ADC, SGR, FCE and PER values of different feeds

Feed	ADC (%)	SGR (%)	FCE (%)	PER (%)
Feed 1	63.18	2.73	33.00	100.62
Feed 2	70.69	3.27	21.00	105.08
Feed 3	64.73	2.61	19.25	95.56
Feed 4	60.56	2.08	20.47	90.43
Feed 5	71.92	3.59	40.44	125.44
Feed 6	72.37	3.45	38.22	115.52

**EFFECTS OF DIETARY LIPID LEVELS AND SOURCES IN ISOENERGETIC DIETS ON
GROWTH, BODY COMPOSITION AND TISSUE FATTY ACID COMPOSITION IN
JUVENILE *Barbodes altus***

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The effects of four (2.5, 5.0, 7.5 or 10.0%) dietary lipid levels of two different sources (squid oil or safflower oils) in isoenergetic diets with the same protein level (42%) on growth and body composition of *Barbodes altus* were examined. At the end of the growth trial, carcass samples were analysed for moisture, crude protein, crude fat, ash, and fatty acids. Increasing the dietary level of lipid with either squid oil or safflower oil as the source, whilst keeping the protein and energy levels constant in the diets, did not affect the growth of *B. altus*. Significantly more lipid was stored in the body of groups fed 10% oil diet with either squid oil or safflower oil as lipid source compared with those fed lower dietary lipid levels (2.5 and 5%), even though all the diets had the same amount of energy. In this present experiment, the significant increase in visceral lipid content and no significant change in muscle lipid content in relation to increased dietary lipid level indicated that, in *B. altus*, excess fat was stored in visceral tissue rather than muscle tissue. In viscera, the concentration of total PUFA at higher levels of safflower oil (7.5 and 10%) was significantly greater than at lower levels (2.5 and 5%). The concentration of total n-6 fatty acids increased in the muscle of fish fed with increasing safflower oil level in the diet. The concentration of total n-3 fatty acids in the muscle of fish was significantly greater when fed at higher levels of dietary squid oil than in fish fed at the 2.5% dietary level.

Table 1 Crude fat content in muscle, liver and viscera of *B. altus* after 8 weeks of feeding isoenergetic diets containing various levels of dietary lipid (squid oil)

Dietary lipid level (%)	As percentage of wet weight		
	Muscle	Liver	Viscera
2.5	2.85±0.32 ^a	9.49±1.92 ^a	16.99±0.48 ^a
5.0	2.58±0.07 ^a	8.61±1.12 ^a	20.12±0.43 ^{ab}
7.5	3.03±0.06 ^a	10.04±2.72 ^a	25.32±2.95 ^b
10.0	3.24±0.22 ^a	9.59±1.92 ^a	24.25±2.08 ^b

Table 2 Crude fat content in muscle, liver and viscera of *B. altus* after 8 weeks of feeding isoenergetic diets containing various levels of dietary lipid (safflower oil)

Dietary lipid level (%)	As percentage of wet weight		
	Muscle	Liver	Viscera
2.5	3.05 ± 0.22 ^a	7.62 ± 0.94 ^a	18.47 ± 3.59 ^a
5.0	3.55 ± 0.25 ^a	8.88 ± 0.77 ^a	23.30 ± 0.66 ^a
7.5	3.28 ± 0.27 ^a	8.09 ± 0.48 ^a	23.09 ± 1.37 ^a
10.0	3.57 ± 0.31 ^a	8.31 ± 0.68 ^a	32.52 ± 3.62 ^b

FEEDING PERIODICITY, GUT EVACUATION RATE AND DAILY RATION OF FINGERLINGS OF MRIGAL *Cirrhinus mrigala* (HAMILTON)

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Feeding periodicity of fingerlings of mrigal, *Cirrhinus mrigala*, was examined from gut collections taken during winter, summer and rainy seasons at the Harangi Reservoir Fish Farm, Karnataka, India. Feeding periodicity was determined by means of mean percent fullness of gut and means gut content weight per gram of body weight of fish. No significant differences in the gut content weight were found between any two successive three-hour sampling periods. The evacuation data were fitted to linear regression model. The evacuation time for natural, conventional and pelleted feeds ranged between 6.95 hr and 9.31 hr. The natural feed evacuated faster than both conventional and pelleted feed. Effects of protein sources, protein and fat level in the diet, starvation period and feeding frequency on evacuation rate in mrigal fingerlings were determined. The daily ration was estimated to be 8.84 %BW to 12.09 %BW with an average value of 10.59 %BW at $28 \pm 1.5^\circ\text{C}$.

Table Gut evacuation time (GET) in mrigal fingerlings.

Type of feed	GER	Time (hr) to percent evacuation		
		25	75	100
Natural feed	0.1932	0.48	4.79	6.95
Conventional feed	0.1822	2.45	7.02	9.31
Pelleted feed	0.1956	2.63	6.89	9.02

ONTOGENETIC CHANGES IN TRYPSIN, AMYLASE AND LIPASE ACTIVITIES IN *Scylla serrata*

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In order to increase productivity in mud crab hatchery, feeding management should be based on information on basic digestive physiology such as ontogenetic changes in digestive enzyme activities. Thus, this study aims to measure the activities of three digestive enzymes, namely amylase, trypsin and lipase in mud crab. Both amylase and trypsin were dominant in the larvae. Tryptic activity showed a smaller peak at Z₂ and a bigger peak at the crab stage. Amylase to total protease (A/P) ratios were determined to be 9.86, 20.4, 5.0, 44.5, 34.3 and 52.2 at larval stages Z1, Z2, Z3, Z4, Z5, and megalopa, respectively. This is an indication that at early larval stages the mud crab was carnivorous and shifts to being omnivorous at the latter stages. It is hypothesized that this is one of several natural strategies towards achieving energetic efficiency in crustaceans (Jones et al. 1997). Low levels of α -amylase at Z1 – Z3 was observed with a sharp increase from Z3 maintained at the maximum all through the megalopa and then decreased at the crab 1 stage. The low amylase activities during early larval stages were another indication that the mud crab larvae are exclusively carnivorous during these periods just like the caridean larvae (Kamarudin 1994). Lipase activities exhibited two similar peaks at Z2 and at Z4-Z5 which is similar to the non-specific esterase ontogenetic pattern in *Macrobrachium rosenbergii* (Kamarudin 1994) which coincided with a sharp increase in hepatopancreas (HP) volume and HP/DW (dry weight) ratio:

FATTY ACID PROFILE IN *Perna viridis* FROM SEBATU, MELAKA

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Fatty acid profiles in green mussel (*P. viridis* L.) with size 5 to 8 cm were analyzed. Samples were taken from Sebatu mussel culture area in Melaka. The objective of this study were to determine fatty acids profile of matured mussel (with gonad) and immatured mussel (without gonad) Analysis was done by using gas chromatography mass spectrum (GCMS). Results showed that mussels have 16 types of fatty acid (FA) with 15 - 23 long chain carbon consisting of five saturated fatty acid, four mono-unsaturated fatty acid (MUFA) and seven poly-unsaturated fatty acid (PUFA). Overall, percentages of saturated fatty acid were highest, followed by PUFA and MUFA (table 1). Percentage of unsaturated fatty acid was the highest especially PUFA in mussel with gonad (matured). Predominant fatty acid in both types of mussel (mature and immature) are hexadecanoic acid (17:0). Ratio of ω -3 to ω -6 for matured mussel was slightly higher than immature mussel which is 8.08:1 and 4.32:1 respectively.

Table 1. Percentages of fatty acids and their ratio between mature and immature mussel.

Fatty Acid	Mussel tissue	
	Immature	matured
Saturated	60.71	57.57
MUFA	15.46	18.3
PUFA	23.83	24.13
Unsaturated	39.33	42.43
Ratio (saturated:unsaturated)	1.54	1.36
ω -3	17.15	14.31
ω -6	3.97	1.77
Ratio (ω -3: ω -6)	4.32	8.08

**ELEVATED DIETARY LEVELS OF TRF, TOCOTRIENOLS-RICH FRACTION,
EXTRACTED FROM CRUDE PALM OIL, AFFECT VITAMIN E STATUS AND OXIDATIVE
STABILITY OF TILAPIA TISSUES**

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A 9-week feeding trial was conducted to evaluate the effects of dietary vitamin E on fish growth, lipid peroxidation and distribution of tocopherols and tocotrienols in various tissues of hybrid red tilapia. Five semi-purified isoenergetic and isonitrogenous diets were supplemented with 0, 139, 278, 556, and 1111 mg/kg, respectively, of a tocotrienols rich fraction (TRF) extracted from crude palm oil. There were no significant differences in fish percent weight gain, feed conversion ratio and protein efficiency ratio among fish given the various test diets ($P>0.05$). Muscle, liver, adipose, skin and plasma concentrations of α -tocopherol, α -tocotrienol, and γ -tocotrienol increased linearly in response to increasing dietary concentrations originating from the added TRF. α -Tocopherol constituted 51.2–94.2% of the vitamin E composition of various tissues. The deposition of tocotrienols was highest in the adipose tissue. The concentrations of thiobarbituric acid-reactive substances (TBARS) from Fe^{2+} -catalysed vitamin C induced lipid peroxidation in muscle, liver and plasma of tilapia fed diets with no added TRF were significantly higher than that found in the tissues of fish fed diets supplemented with TRF. Results obtained from the present study indicated that TRF supplementation could markedly enhance the tocotrienols concentration in various tilapia tissues and provide higher protection of these tissues against lipid peroxidation.

EVALUATION OF MIXED FEEDING SCHEDULES WITH VARYING DIETARY PROTEIN CONTENT ON THE GROWTH PERFORMANCE AND REDUCTION OF COST OF PRODUCTION FOR PANGAS, *Pangas hypophthalmus*, WITH SILVER CARP, *Hypophthalmichthys molitrix*, IN FARMERS PONDS

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A 6-month growth trial was conducted under field conditions using fish farmers' ponds to investigate the suitability of mixed feeding schedules on the growth performance and production costs for pangas, *Pangas hypophthalmus*, with silver carp, *Hypophthalmichthys molitrix*. Ten farmers' ponds (10-15 dec) were used for the study. Fish were stocked at the ratio of 80: 20 (pangas: silver carp) at the total rate of 25,000 / ha. The mean initial weights of fish were 4.9 and 12.0 g for pangas and silver carp, respectively. Two diets- high-protein (30%, HP) and low protein (15%, LP) were prepared by using locally available ingredients and fed to the fish continuously served as the control treatments. Five different feeding schedules of high protein (control-1, HP), low protein (control-2, LP), 1 day low protein followed by 1 day high protein diet (1L/1H), 7 days low and 7 days high protein diet (7L/7H) and 14 days of low followed by 14 days of high protein diet (14L/14H) were tested. The fish were fed twice daily at the rate of 15, 10, 8 or 5% of their body weight for the 1st, 2nd, 3rd and subsequent months of the experimental period, respectively. Feeding rate was calculated only on the basis of pangas weight and was adjusted after fortnightly weighing of fish. Fishes fed low protein and high protein diet on alternate days (1L/1H) resulted in significantly ($P < 0.05$) higher growth rate, feed utilization and production among the treatments. The FCR values of pangas ranged between 2.04 to 2.79 with feeding schedule 1L/1H showing significantly the lowest i.e. the best FCR. The FCR values were relatively lower when weight of silver carp was considered which ranged between 1.61 to 2.04. There was no significant difference ($P > 0.05$) between the growth rates and production of fish fed high protein diet (30%, HP) regularly and fish fed 7 days low protein diet followed by 7 days high protein diet (7L/7H). Fish maintained on low protein diet (15% LP) grew least. The total production of fish (including silver carp) ranged between 8,310 to 12,422 kg/ha with fish on the 1L/1H feeding schedule resulting in the highest production and net profit. The present study reveals that feeding fish continuously with a high protein diet is uneconomical. It is recommended that for profitable pangas culture with silver carp in Bangladesh, farmers can use a mixed feeding schedule of alternate day feeding of low and high protein diets as a means of reducing feed cost.

Table 1 Final weight (g), production (kg/ha) and net profit (Tk/ha) of pangas and silver carp maintained on mixed feeding schedules for 6 months in farmers ponds

Parameters	Feeding schedules				
	HP	LP	1L/1H	7L/7H	14L/14H
Mean initial weight (pangas)	4.9	4.9	4.9	4.9	4.9
Mean initial weight (silver carp)	12.0	12.0	12.0	12.0	12.0
Mean final weight (pangas)	495.0 ^b	350.0 ^d	520.0 ^a	476.0 ^b	421.0 ^c
Mean final weight (silver carp)	552.5 ^{ab}	490.0 ^c	590.0 ^a	533.0 ^{bc}	510.0 ^{bc}
Production (pangas)	8945.0 ^b	6123.0 ^d	9722.0 ^a	8421.5 ^b	7329.5 ^c
Production (silver carp)	2406.0 ^{ab}	2187.6 ^b	2700.5 ^a	2467.5 ^{ab}	2328.3 ^b
Total production (kg/ha)	11351.0 ^b	8310.6 ^d	12422.5 ^a	10888.9 ^b	9657.8 ^c
Net profit (Tk/ha)	1,05,619	81,523	1,59,954	1,15,982	87,316

Tk. 58.00 = US \$ 1.00

* Figures in the same row having different superscripts are significantly different ($P < 0.05$)

**EVALUATION OF THE EFFECT OF DIETARY VITAMIN C, E AND ZINC
SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE OF NILE TILAPIA
*Oreochromis niloticus***

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A 3×2 factorial experiment was conducted to evaluate the role of dietary vitamin C (ascorbic acid) and vitamin E (α -tocopherol) and zinc supplementation on *Oreochromis niloticus* growth, survival and reproduction in hapa based system installed in earthen ponds for 5 months. The triplicate groups consisting 30 fish were fed three levels of vitamin C (0, 50 and 1250 mg/kg diet), two levels of vitamin E (0, 600 mg/kg diet). An additional treatment that supplemented zinc at a rate of 120 mg/kg with higher vitamin C and E levels was also included in the experiment. Vitamin C deficient fish exhibited significantly lower daily weight gain and percentage weight gain compared to the supplementation of higher vitamin C in the diet ($P<0.05$). The spawning efficiency, total seed production and number of eggs/spawner were not affected by dietary vitamin C or E supplementation. However, the spawning percentage, total number of spawns and total seed production in hapa (80.0 ± 28.28 , 26 ± 6 and $10,991\pm1334$, respectively) were higher in fish fed the dietary zinc supplemented diet. The brood fish fed diets supplemented of with 1250 mg/kg vitamin C and 600 mg/kg vitamin E had a higher mean clutch size and relative fecundity (626 ± 80 and 7462 ± 588 , respectively). The fertilization rate, egg hatchability and larval survival were 76.19 ± 5.58 and 88.89 ± 4.68 , 19.13 ± 8.56 and 45.92 ± 6.33 , 57.74 ± 15.02 and 81.09 ± 2.93 , respectively, for females fed on diets without supplementing vitamins and diet supplemented with higher levels of vitamin C and E, -. There was a high correlation between gonadal ascorbate content and α -tocopherol concentration in diets. Fish fed diet without ascorbic acid had reduced sperm viability and sperm motility compared with supplementation of 1250 mg vitamin C/kg in the diet. No significant positive effects were observed on egg quality or sperm quality of *O. niloticus* fed higher zinc level in the diet. Present findings suggest that the dietary vitamin C supplementation of 50 mg/kg diet and 120 mg/kg Zinc in the diet support maximum egg production, egg quality and sperm quality. Higher vitamin C levels (1250 mg/kg) in the diet may not be necessary to enhance seed production.

**ESTIMATION OF IN VITRO PROTEIN DIGESTIBILITY AND INHIBITORY EFFECTS OF
DIFFERENT FEED AND INGREDIENTS ON PROTEASE ACTIVITY OF FRESH WATER
GIANT PRAWN *Macrobrachium rosenbergii* (DE MAN)**

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Rapid and inexpensive technique for estimating protein digestibility and inhibitory effect of feed and its ingredients are *in vitro* enzyme assay. Two assays – Lazo single enzyme assay using bovine trypsin and pH-drop method using prawn crude enzyme were used to measure the relative protein digestibility (RPD) of different feed (seven formulated diets) and feed ingredients namely fishmeal (FM), extracted soybean meal (ExSM), mustard oil cake (MOC), sesame oil cake (SOC) and coconut oil cake (COC) used in diets of freshwater giant prawn, *Macrobrachium rosenbergii*. pH-drop method using prawn crude enzyme appears to give better indication in RPD determination of *Macrobrachium rosenbergii*.

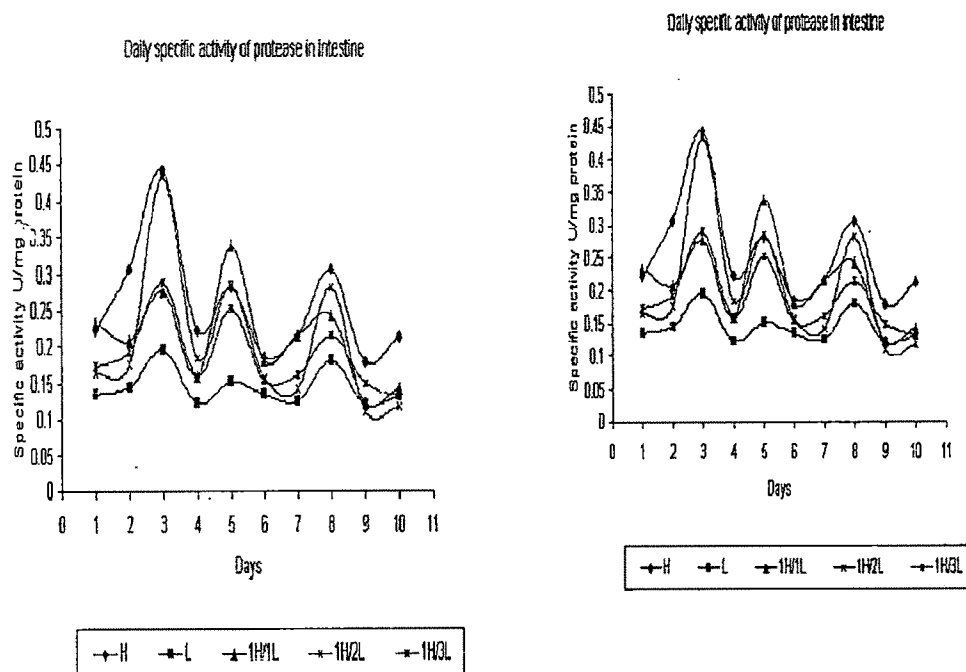
Further, the effect of protease inhibitors present in different feed ingredients used in feed for *Macrobrachium rosenbergii* were assayed using *in vitro* technique involved in estimation of the remaining protease activity after incubation with different solutions containing inhibitors. Inhibitors obtained from extraction of plant proteins (ExSM, MOC, SOC and COC) produced 5 to 56% of inhibition on the total protease activity and that of animal protein (Danish Fishmeal) produced 4% of inhibition on the total protease activity. The use of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) zymograms allowed visualization of the aforementioned differences in inhibition.

THE EFFECT OF MIXED FEEDING SCHEDULES ON THE DAILY SPECIFIC ACTIVITY OF PROTEASES IN STOMACH AND INTESTINE OF AFRICAN CATFISH *Clarias gariepinus*

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An experiment was conducted to evaluate the effect of mixed feeding schedules on the daily specific activity of proteases in the stomach and intestine of the African catfish *Clarias gariepinus*. Two practical diets containing 35% (high) and 25% (low) protein levels containing 10% lipid level were prepared. The three mixed-feeding schedules tested were as follows: one day of 35 % protein followed by one day of 25 % protein (1H: 1L); one day of 35 % protein followed by 2 consecutive days of 25 % protein (1H/2L); and one day of 35 % protein followed by 3 consecutive days of 25 % protein (1H/3L). Four hundred and fifty catfish ranging from 300-400 g were stocked in 15 circular 1m³ fiber tanks at a rate of 30 fish /tank with three replicates per treatment. The fish were fed three times daily to satiety for a period of 10 days. Daily specific activity of proteases in the stomach and the intestine were sampled by collecting three fish from each replicate 4hrs after feeding. Results show the existence of a daily rhythmic pattern regardless of feeding schedule and that fish fed the 35% protein diet had a higher protease specific activity compared to those fed the 25% protein diets. Generally, a trend of one day of high followed by one day of low protease specific activity was detected in the stomach and intestine.



THE EFFECT OF DIETARY INCLUSION OF SELECTED SOY PRODUCTS AS PARTIAL REPLACEMENT FOR FISH MEAL ON THE GROWTH AND FEED UTILIZATION OF *Oreochromis niloticus*

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Five experimental diets were used to evaluate the effect of utilizing selected soy products as protein sources on the growth and feed utilization of *Oreochromis niloticus*. Protein sources, solvent-extrated soybean meal (SESM), roasted soybean meal (ROSM), raw soybean meal (RSM) and boiled soybean meal (BSM) were incorporated approximately at 10% level into isonitrogenous (30% protein) diets A, B, C and D respectively, to replace fish meal (FM) in a control diet (Diet E). Young male *O. niloticus* with mean initial weight of 10.98 g were fed on the experimental diets in a seven-week feeding trial in aquaria. The results of the study showed that fish fed ROSM diet produced the best weight gain (9.83 g fish⁻¹), specific growth rate (1.31% day⁻¹), protein efficiency ratio (0.98) and feed conversion efficiency (29.5 %). However, the data showed that inclusion of selected soy products at 10% in tilapia diet had no significant effect ($P>0.05$) on the growth performance, feed utilization and survival compared to fish fed Diet E. The dietary soy products did not affect the hepatosomatic indices (1.17-1.81%) ($P>0.05$).

Among the five tilapia diets, ROSM diet proved to be the best in terms of economic analysis. The results suggest that SESM, ROSM, RSM and BSM are suitable ingredients in tilapia diet at a level of 10%. Future studies should be directed to assess using these soy products as feed components at higher inclusion levels and to further anticipate doing feeding trials under pond culture conditions so as to clarify the results observed from the present study.

Table 1 Growth, feed utilization and hepatosomatic index (HSI) of *Oreochromis niloticus* fed with soy product ingredients and control diet. Means with the same superscripts within a column are not significantly different ($P>0.05$).

Diet	Average body weight (g)		Weight gain (g)	Specific growth rate (%)	Feed efficiency (%)	PER	HSI (%)
	Initial	Final					
A	10.90	20.21 ^a	9.31 ^a	1.23 ^a	29.32 ^a	0.97 ^a	1.65 ^a
B	10.86	20.69 ^a	9.83 ^a	1.31 ^a	29.52 ^a	0.98 ^a	1.66 ^a
C	11.04	17.61 ^a	6.57 ^a	0.95 ^a	20.82 ^a	0.70 ^a	1.59 ^a
D	11.08	17.69 ^a	6.61 ^a	0.95 ^a	20.76 ^a	0.69 ^a	1.17 ^a
E	11.05	20.02 ^a	8.97 ^a	1.20 ^a	26.39 ^a	0.87 ^a	1.81 ^a

BREEDING CAPABILITY OF THE HYBRID CATFISH *Clarias macrocephalus* X *C. gariepinus* IN CAPTIVE CONDITION

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The study was conducted in order to assess the breeding capability of the hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*). Hybrid catfish (Hybrid 1) was produced artificially and reared in 3-ton fiberglass tanks at the density of 20 fish per square-meter. They were fed with tilapia starter pellet (34% protein) at a daily rate of 2% of fish biomass. Female of hybrid 1 can reach first maturation in captive condition at the size of 0.38 ± 0.08 kg (11 month old). 72.89% (n = 107) females obtained full maturation and can be induced to spawn. The remaining female fish had an abnormal gonad. Artificial breeding among F1 hybrids was not successful probably due to a problem of spermatogenesis. Whereas, mature hybrid females were successfully backcrossed with both parental species and hybrid 3 (female hybrid 1 x male *C. macrocephalus*) and hybrid 4 (female hybrid 1 x male *C. gariepinus*) were produced. The fertilization rates of the hybrid 3 and hybrid 4 were 73.98% and 72.01%, respectively. However, hatching rates were low, 62.81% and 60.32%, and deformation rates were high, 17.32% and 25.58%, for hybrid 3 and hybrid 4 respectively, compared to those of parental species. Results of larval rearing showed that in the first 30 day after hatching, the growth rate of backcrossed hybrid was comparable to those of *C. gariepinus* and higher than *C. macrocephalus* with specific growth rate (SGR) of 16.84 and 17.89%.day⁻¹ for hybrid 3 and hybrid 4 respectively.

INDUCED BREEDING AND LARVAE REARING OF *Ompok pabda* (HAMILTON)

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Ompok pabda (Hamilton) is an important species of Bangladesh and commercial culture of this species is not possible because of lack of seed for stocking. Successful induced breeding and larval rearing of *O. pabda* have not been reported. Therefore, this study attempts to optimize the doses of two inducing agents (PG and HCG) to achieve spawning success and also to increase the larval survival by determining the suitable frequency of food delivery. Four single PG doses of 14, 16, 18 and 20 mg/kg bw of female were tested; males received 12mg/kg. The PG dose of 18 mg had a 67% spawning 88% fertilization 76% hatching success. In a second experiment HCG was given in 4 single doses (0.5, 1.0, 1.5 and 2.0 IU/g). Males were given 0.5 IU/g. The dose of 0.5 IU/g bw failed to induce spawning. Among the rest, the dose of 1.0 IU/g bw had a better result compared to other two doses with a 50% spawning, 78% fertilization and 60% hatching.

In the feeding frequency study, 5 days old larvae produced in induced breeding trial, were reared for a period of 21 days with live feed, Tubificid worms. Larvae were fed to satiation under the feeding frequencies of once, twice, three times and four times per day. Higher percent length, weight gain, and specific growth rate were shown by the larvae fed four times a day followed by those fed three times, twice and once daily. The highest survival rate (81.5%) was found among those at feeding frequency of four times per day whereas the lowest (66.25%) was found in those fed once a day. The study thus, not only demonstrated the optimum doses of two widely used inducing agents but also a suitable feeding frequency for larval survival and growth.

Effects of different doses of PG on induced breeding of *O. pabda* expressed in terms of spawning rate, fertilization rate and hatching rate. Values are mean $\pm$ standard deviation.

PG (mg/kg)	No. of fish (pair)	No. spawne d	% Spawn	Fertilization (%)	Hatching (%)
14	17	4	24	70.96 ^b $\pm$ 4.06	54.60 ^b $\pm$ 8.67
16	17	7	41	81.82 ^{ab} $\pm$ 3.21	64.71 ^{ab} $\pm$ 5.98
18	21	14	67	87.89 ^a $\pm$ 6.67	76.21 ^a $\pm$ 4.72
20	26	9	35	59.32 ^c $\pm$ 7.23	36.59 ^c $\pm$ 5.74

*Figures in the same column having different superscripts are significantly ($p < 0.05$) different

EFFECT OF TRANSPORTATION STRESS ON THE FEEDING OF MAJOR CARP HATCHLINGS IN A NURSERY POND OF BANGLADESH

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It is important that the carp larvae have developed the habit to feed before they are released in to the nursery pond. The question raised in developing the present research is whether major carp (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) fry have any difficulty in searching for food in nursery pond when they spend their early feeding stage in plastic bags (PB) or aluminum pitcher (AP) during short (5 hours) or long term transportation (12 hours). In addition to non-feeding habit, transportation-stress could result in mortality. Initially, it was found that the carp hatchlings do not feed just after releasing in to nursing pond (Table 1). This was due to transportation stress. However, 50% of hatchlings/fry after transported in PB and AP exhibited active feeding within half-an-hour. Hundred percent hatchlings/fry started active feeding after 5 hours of release in nursery pond. The survival of fish fry is about 60% in the nursery during this experiment. There is no risk in transporting fish hatchlings in oxygenated bags even with partial yolk sac. However, in traditional pitcher with water hyacinth, the survival of hatchlings was (about 50% mortality) than the oxygenated plastic bags. The water quality degradation and injury due to agitation by hand flapping may have resulted in higher mortality in aluminum pitcher.

Table 1 Food acceptance of carp fry in different time intervals (5min, 30 min, 1 hour, 3 hours and 5 hours) in nursing hapa after transportation in oxygenated plastic (OP) bags and aluminum pitcher (AP).

Species (mortality%, if any)	Transportation simulation (Type/ hrs)	05 min	30 min	Food in stomach (%) after 1 hrs	3 hrs	5 hrs
<i>Catla catla</i>						
0	OP (5hrs)	0	18.67±1.76	48.33±3.38	73.33±4.18	100
32	AP (5 hrs)	0	9.67±.88	49±4.58	76±4.93	100
18	Control 5 hrs	77±1.15	100	100	100	100
0	OP (12hrs)	0	12±1.73	51±4.73	67.67±5.55	90.33±.88
53	AP (12 hrs)	0	7.33±1.2	34.33±6.89	62±3.51	100
26	Control 12 hrs	80.33±3.93	100	100	100	100
<i>Labeo rohita</i>						
0	OP (5hrs)	0	21±2.08	58.33±3.84	80.33±5.21	100
38	AP (5 hrs)	0	13±1.53	51.33±5.46	72.67±4.41	100
16	Control 5 hrs	73±4.93	100	100	100	100
0	OP (12hrs)	0	24±5.86	47.67±2.03	74.67±4.37	100
46	AP (12 hrs)	0	8.67±1.2	42±5.2	68±5.2	100
12	Control 12 hrs	81±2.08	100	100	100	100
<i>Cirrhinus mrigala</i>						
0	OP (5hrs)	0	39.33±3.48	48.33±3.38	99±.58	100
18	AP (5 hrs)	0	30.67±2.85	49±4.58	91.67±2.03	100
12	Control 5 hrs	87.33±.67	100	100	100	100
0	OP (12hrs)	0	25±1.73	51±4.73	94.33±2.91	100
20	AP (12 hrs)	0	22.67±.67	34.33±6.89	82.33±2.6	100
10	Control 12 hrs	81±3.61	100	100	100	100

EFFECT OF HOLDING TEMPERATURE AND LIGHT REGIME ON REPRODUCTIVE PERFORMANCE OF RAINBOW TROUT *Oncorhynchus mykiss* IN THAILAND

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In Thailand, the rainbow trout (*Oncorhynchus mykiss*) was introduced by His Majesty King Bhumibol Adulyadej of Thailand in 1969 to induce trout farming in the northern highlands as a cash income source for the hill tribe people to enable them to abandon opium poppy cultivation. Compared to other salmonids, rainbow trout are tolerant to relatively high water temperatures but there are only very limited information available about the effects of elevated temperatures on the reproductive success. Reproductive development of salmonid fish is known to be strongly influenced by photoperiod but till now investigations on reproductive performance of rainbow trout under the photoperiod and the ambient water temperatures in the highlands of northern Thailand are missing.

Fifteen months old rainbow trout, raised under ambient water temperatures and natural light regime at the Doi Inthanon Fisheries Research Unit since hatching, were divided randomly into three groups (March). The first group (105 fish) was exposed to a light programme, which consisted of the natural light regime of Germany with an increasing photoperiod till June and thereafter a decreasing photoperiod till December. Water temperature was controlled in such a way that it did not exceed 18°C. Fish of the second group (105 fish) were kept under ambient water temperatures but the same light regime as group 1 fish. Fish of the third group (420 fish) were kept under ambient water temperatures (mean temperatures of 14.6°C in January and 20°C in May) and the natural photoperiod (11 to 13 hours of daylight the whole year round) of Northern Thailand. At the age of 23 months (body weight of female spawners between 0.9 and 1.4kg), brood stocks started to spawn. Spawning season lasted from November to January with concentration of spawning events (number of fish with ovulated eggs) in December. Eggs from nearly all females in group 1 could be obtained before January, whereas the percentage of females with ovulated eggs was reduced to 84% in group 2 and to 68% in group 3. In these two groups ovulated eggs were obtained also in January. The percentage of fish with overripe eggs was on average lowest in group 1 with 8.8% compared to 27% in both other groups. The weight of female spawners was significantly lower in group 3 (1kg) than in the two other groups (1.3kg). On average, the number of ovulated eggs per kg body mass of spawners was reduced from 1357 in group 1 to 1136 in group 2 and to 1185 in group 3 with significantly lower egg diameters of 4.45mm in group 2 and in group 3 compared to 4.67mm in group 1. With 75%, the fertilization rate was highest in group 1, followed by 55% in group 3 and 44% in group 2. Survival rates till the eyed egg stage showed the same ranking with rates of 43% in group 1, 29% in group 3 and 20% in group 2. For hatching rates, on average 24%, 20%, 25% in group 1, 2 and 3 respectively, no significant differences could be found between treatment groups. The best hatching rates obtained in group 3 were in the range of 78 to 87%. It has to be seen if and how much reproductive performance can be improved further, when spawners of the second generation will come into use.

IMPROVED LOW COST INCUBATION SYSTEM TO INCREASE HATCHING AND SURVIVAL OF LARVAE FROM NATURALLY SPAWNED EGGS OF INDIAN MAJOR CARPS IN HALDA RIVER

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This study compared various locally made incubation systems to increase hatching and survival rates of eggs collected from Haldia River in Bangladesh for enhancing the quality wild seed. Six different types of incubation systems with circulation and without circulation of pond and river water with different proportion of eggs in different systems were tested. Ponds dugout on the riverbank, a traditional incubation system for river-collected eggs of Indian major carps has low hatching and survival rates. Highly significant variation ($P < 0.05$) existed among the hatching rate of different incubation systems except between the Plastic tank and Tiles cistern where hatching rates were the highest ($> 90\%$). All incubation techniques with circulation of water resulted in significantly higher hatching and survival rate than the non-circulated water in cloth hapa and earthen pond. However, hatching in Galvanized Iron sheet bottle with circulation of water caused sand particles coating the eggs and hatchlings resulting in low hatching and high mortality. Quality of both river and pond water (temperature, pH, dissolved oxygen) showed no major variation except the secchi disc visibility which was much lower in river water due to turbidity. Comparison between six incubation systems clearly indicated that plastic tank and tile cistern were far superior to other systems compared. By increasing hatching and survival rate by as much as 50%, this study therefore, contributes to enhance wild seed production by the poor seed collectors.

Table1 Efficiency of six different incubation systems for hatching of naturally spawned fertilized eggs of major carps from the River Halda, Chittagong, Bangladesh. Means in the last column followed by different superscript letter differ significantly (ANOVA, DMRT; $P < 0.05$)

Six different types of incubation system (Hatchery)			Nos. of Fertilized Eggs in different systems	Nos. of fertilized Eggs hatched in different systems (hatching rate %)	Mean hatching and survival rate (%)
1	Plastic Tank	1	18,609	16,050 (86.25%)	90.99 ^a
		2	22,414	21,600 (96.36%)	
		3	76,672	69,283 (90.36%)	
2	G.I. Sheet Bottle	1	41,165	6,300 (15.30%)	15.30 ^e
		2	39,165	6,000 (15.31%)	
		3	47,150	7,211 (15.29%)	
3	Tiles Cistern	1	1,22,820	1,14,750 (93.43%)	94.48 ^a
		2	1,56,316	1,49,000 (95.32%)	
		3	2,19,326	2,07,700 (94.69%)	
4	Cloth Hapa	1	1,13,510	49,059 (43.22%)	44.35 ^d
		2	1,09,000	48,232 (44.25%)	
		3	1,06,900	48,746 (45.60%)	
5	Earth pond	1	12,34,000	6,22,000 (50.40%)	48.87 ^c
		2	13,50,250	6,42,000 (47.55%)	
		3	13,10,150	6,30,000 (48.67%)	
6	Cement cistern	1	2,17,604	1,75,000 (80.42%)	79.81 ^b
		2	2,05,000	1,60,000 (78.05%)	
		3	2,28,500	1,85,000 (80.96%)	

NURSING OF HATCHERY PRODUCED *Penaeus monodon* POST-LARVAE (PL) IN FLOATING CAGES AS AN ALTERNATE LIVELIHOOD OPTION IN BANGLADESH

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Shrimp post larvae (PL) collection is a primary income generating activity among the poor coastal people of Bangladesh. They are called PLs collector and the only suppliers of wild PL to the shrimp culture farms. Collection of wild PL is a primary income generating activity of PL collectors. During collection of PL from the wild they destroy other shrimps and fish fry as by catch. It is estimated that for one *P. monodon* PL collected, 99 fry of other fish and shrimp are destroyed. As a result natural resources are being declining sharply. To overcome this problem government of Bangladesh has recently banned PL collection, affecting the livelihoods of PL collectors.

Nursing hatchery produced *P. monodon* PL could be an alternative and suitable economic activity for the livelihood of such PL collectors. Stocking nursed PL in the culture pond as opposed to direct stocking of hatchery PL, enables farmers to more accurately predict survival, standing crop, feeding rate and production level in grow out ponds. Nursing could be in ponds, hapas or in floating cages. It was assumed that floating cage nursery could be most appropriate and suitable for PL collectors because cages can be installed in sheltered open water along the coast where they have free access.

Nursing for *P. monodon* post-larvae in floating cages of 2 x 3 x 1.5m, installed in sheltered areas of the coast were tried. Cage frame was made from locally available wood or bamboo. Two nets one as outer and other as inner net were fixed to the cage frame. Four empty plastic drums of 150l capacity were used as floats. Post-larvae PL₁₀ to PL₁₅ purchased from the hatchery were stocked in the cages at a density of 2-4 PL/l. Raw ground fish or dry fish meal were provided as feed 3-4 times a day. Average survival rate after 6-7 days nursing was 80% with promising growth. Two to seven fold growths were recorded in 6-7 days nursing period. The system was found as profitable alternate livelihood option for PL collectors.

PROPAGATION OF FIRST GENERATION (F1) OF *Pangasianodon hypophthalmus*

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Pangasianodon hypophthalmus has been bred in 1990 at Bati Fish Seed Production and Research Station in Cambodia from 30 pairs of wild brood fish that were collected from Mekong River in Prey Veng Province. Thirty pairs of brood fish were stocked in 300 m² pond at a stocking density of 1 fish/5 m². The brood fish were fed with pellet feed containing 35% protein. Water quality was monitored and water was exchanged.

The female fish matured within 3 years plus, but male fish matured at the third year only. Their weight ranged from 4-7 kg.

The artificial propagation of these brood fish was conducted with dry method. The hormone for female fish was administered with HCG at a priming dose of 500 IU/kg and 20 µg/kg of Suprefact plus 5 mg/kg of Domperidon as resolving dose. Male fish were injected with half the resolving dose of female. The ova stickiness was removed by using Tanin Acid solution at 1 mg/liter of water.

The study showed that the duration of fish maturation under culture conditions is one year shorter than wild brood stock. The cultured fish accepted the supplementary feed better than the wild brood fish. It was easier to spawn cultured fish than the wild brood stock. Gonad weight ranged from 4 to 15% of fish body weight.

TILAPIA BREEDING IN TANK TO ENHANCE SEED PRODUCTION

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Research has been conducted to breed red Tilapia in tank at Freshwater Fisheries Research Centre, Batu Berendam, Melaka, Malaysia. A 3.6m x 2.7m x 0.5m concrete tank was used. The tank was filled with the domestic water supply to a depth of 0.3 m. The experiment was carried out with minimal water exchange. The tank was continuously supplied with air and the oxygen level was maintained above 3.5 ppm. After a week, 20 brooders of 250g each comprising 5 male and 15 females were introduced into the breeding tank. Fish were fed once a day with starter floating tilapia diet at the rate of 1.0% biomass. The waste from the tank bottom was siphoned out as and when necessary. Fish larvae were seen to be released from the mouth of the brooders at day 15 after stocking. The larvae were then scoped with hand-net three times daily at 0800 hr, 1200 hr and 1600 hr. The larvae were counted individually and recorded prior to nursing. After 55 days breeding duration, 30,484 larvae (0.5 mm) were collected. An average of 40 larvae per day was found to be produced from a single female brooder. From this experiment, it was concluded that tilapia breeding in tank has a potential to enhance seed production.

PRELIMINARY RESULTS OF INDUCED SPAWNING AND NURSING OF *Cirrhinus microlepis* IN THE MEKONG DELTA, VIETNAM

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Cirrhinus microlepis is of great interest in Lao PDR, Thailand and Vietnam. Artificial propagation of this fish started in the Mekong Delta of Vietnam 2001, as part of the Aquaculture of Indigenous Mekong Fish Species (AIMS) Component, Mekong River Commission (MRC). The first larvae and fingerlings were obtained in 2004. Brood stock conditioning, induced spawning and nursing techniques have initially been established. Preliminary results of artificial propagation of *C. microlepis* are discussed, and plans for optimising induced spawning techniques are presented.

CULTURE OF COPEPODS FOR MARINE FINFISH HATCHERIES

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Rotifers and brine shrimp nauplii are the two common live food organisms that have been used to successfully raise hundreds of marine finfish species in hatcheries. On the other hand, several desirable marine finfish species suffered high mortalities when copepod nauplii were not available at the time of first feeding. Besides their size, copepods have other desirable characteristics, such as their nutritional value to finfish larvae. Some copepod species provide greater amounts of the highly unsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) than rotifers or brine shrimp. So far, there has only been limited information about copepod culture scattered in diverse sources. A mass production technique for copepods has yet to be developed. An international workshop was held in Hawaii in 2003 to gather information about the status of culturing copepods in hatcheries and basic biological studies on various types of copepods.

At the workshop, in addition to aquaculture biologists, copepod biologists and ecologists were invited to present their work and discuss key issues related to copepod culture. The topics of the workshop covered the current culture status of copepods and basic biology, such as the production of resting eggs, reproductive performance, behavior, and natural productivity of various types of copepods. It is hoped that these studies and the knowledge gained thereby will help to advance the techniques for culturing copepods and applying them to larviculture.

All of the information presented at the workshop and a summary of the discussion sessions are to be published by Blackwell Publishing this year. This presentation will summarize the information presented and discussed at the workshop.

THE COMPARISON OF NATURAL AND ARTIFICIAL FROG *Rana rugulosa* BREEDING

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The objectives were (1) to compare the spawning and hatching rate in natural and artificial breeding and (2) to compare the economic return from each method. The results are based on three trials. Spawning from artificial breeding resulted in 33,000 eggs while from natural breeding it was 26,000 eggs. Hatching rates from both methods were not different. After one month nursing (tadpole period) the survival rate from natural breeding was higher (60%) than artificial breeding (40%). The development to adult frog was 40% from natural breeding while from artificial breeding it was 30%. The survival rates of frog from month old - 2 month old were not much different.

SOME ASPECTS OF REPRODUCTIVE BIOLOGY AND REARING OF YOUNG STAGES OF *Belontia signata* (GUNTHER)

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Belontia signata (Gunther) is a threatened endemic fish of Sri Lanka, which is also named as a conservation dependent species in the IUCN Global Red Data List. This study was aimed to develop captive breeding and rearing the young of *B. signata* in order to reintroduce the captive bred fish to the habitats where the population has already declined.

Breeding was possible under indoor conditions without hormonal stimulation. They were serial as well as seasonal spawners and spawn 5 – 6 times per spawning season under captive conditions at 26 – 28 °C. Fecundity was 190 – 322 eggs / spawning with an egg size of 956 – 1042 µm. Duration of development stages were egg to hatchling within 48 hr, free swimming stage in 3 – 4 days, juvenile stage by 21 – 30 days. Males attain a total body length of 8.2 – 10.9 cm and females of 5.4 – 7.5 cm by one year under captivity.

B. signata fry were tested with three feeding regimes, *Artemia* nauplii, microworm (*Anguillula* sp.) and formulated feed for 6 weeks. Fry fed with *Artemia* nauplii exhibited the highest growth ($P < 0.05$) compared with microworm fed fry. Formulated feed was not suitable for rearing the fry as 100% mortality was observed within 14 days. There was a significant interaction ($P < 0.05$) between the time and growth performance, which revealed that microworm was more suitable during the first three weeks than *Artemia* nauplii.

Four types of test feeds, wild guppy (*Poecilia reticulata* (Peters)) at a length of 6- 8mm, raw swine liver, dried swine liver and formulated feed were used for juveniles for a period of 6 weeks. Guppy fed juveniles showed significantly highest growth performance ($P < 0.05$) when compared with the formulated feed and dried swine liver. There was no significant difference ($P > 0.05$) between the growth performance of formulated feed and dried swine liver fed juveniles. Raw swine liver found to be unsuitable for the juveniles as they did not survive for more than 14 days. Swine liver is cheap (Sri Lankan rupees 25 per kg) and used only for dog meat in Sri Lanka and the results showed that the dried form of swine liver has a good potential in raising the *B. signata* juveniles under captive conditions.

STUDIES ON SEED PRODUCTION OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* IN A BACKYARD HATCHERY

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Maharashtra, the third largest state of the Indian union having rich resources for prawn farming in terms of water spread area. The water bodies available for culture based capture fisheries is estimated at 3.0 lakh hectares which includes ponds, lakes, tanks and reservoirs. A portion of more than 50,000 hectares of unproductive saline sugar cane land of Western Maharashtra offers a very good potential for freshwater prawn farming. Despite tremendous possibilities, freshwater prawn culture is lagging behind in the state. Non-availability of sufficient quality seed is one of the main constraints in the development of freshwater prawn culture in the State. Mostly the seed requirement for the culture is sufficed with the natural collection from the rivers and that too with a meager quantity. As the awareness of freshwater prawn culture among the aquaculturists has increased during the last decade or so, the demand for quality prawn seed has increased many folds. Estimated prawn seed demand will be more than 75 to 100 crores from the State if only 25% of the total potential area have been brought under freshwater prawn culture. Hence, there is a vast scope to establish more than 50 backyard hatcheries of 2.0 million seed production capacity each in coastal region of the State. Even though the technology of seed production has developed, there was no commercial freshwater prawn seed hatchery through out the State till 2001. Recently, a backyard hatchery was established in Ratnagiri district with only 5.0 million seed production capacity under the guidance of Faculty of Fisheries, Dr. B.S. Konkan Agricultural University. This itself is a big achievement, considering the fact that the entrepreneur is a lady in a remote rural village in Ratnagiri district. During hatchery operations, it was observed that generally first post-larva appeared on 16 - 17th day with a total period of 30 to 32 days for completion of larval cycle and more than 80% survival from first zoeal stage. In this paper the details about hatchery facilities, seed production technology, results of seed production, future demand and successful story of a backyard hatchery, which is run by a first woman entrepreneur in the State, is discussed.

REMOVAL OF EGG STICKINESS IN AFRICAN GIANT CATFISH *Heterobranchus bidorsalis*

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The African giant catfish, *Heterobranchus bidorsalis* is an important food fish in Africa and is widely cultivated under various culture systems. *H. bidorsalis* eggs develop a thick gelatinous outer layer, which is very sticky immediately after fertilization and on contact with water. Stickiness is one of the major problems in incubation of *H. bidorsalis* eggs. The removal of "egg sticking" is therefore an essential factor for artificial reproduction and successful incubation of eggs. This study aims at providing a better understanding of the method of eliminating stickiness of *H. bidorsalis* eggs for greater hatchability, survival and yield of larvae, and to ascertain the use of NaCl /urea solution for removing stickiness.

1200-eyed eggs of *H. bidorsalis* were randomly distributed into 12 incubation trays at 100 eggs per tray. Four treatments: combination of urea and sodium chloride at 4gNaCl/3g urea per litre (treatment 1); 3g/l urea solution only (treatment 2); 4g/l NaCl solution only (treatment 3); and aerated water (treatment 4 control), were tested in triplicate incubation trays containing eyed eggs. The eyed eggs were washed in each solution for 45mins. The following parameters were measured: egg stickiness removal was measured by observing the rate at which eggs were detached from one another in each solution and the control, time of hatching and period (the time between the onset of hatching and the point at which no further hatching take place) and the time of onset of yolk absorption and the time at which all the fry had fully absorbed their yolk were recorded for each solution, % hatching, % larvae deformity and % larvae survival.

Detachment of eggs from one another started at 30, 5, 15 and 25 minutes for treatment 1, 2, 3 and 4, respectively. The effects of pre NaCl/urea treatments on hatching and survival of *H. bidorsalis* is shown in Table 1. The hatching periods, % hatching, % larvae deformity and % larvae survival were affected by treatments of eggs with NaCl/urea solutions.

Table 1 Effect of NaCl/urea solution on hatching and survival of *H. bidorsalis* larvae.

Treatment	Hatching period (hrs.)	% Hatching	% deformed larvae	% survival of larvae
3g urea	45.00±2.00 ^a	18.00±0.00 ^c	8.00±0.00 ^a	10.00±0.00 ^c
4gNaCl/3g urea	40.00±1.50 ^b	80.00±4.00 ^a	4.00±0.02 ^b	76.00±2.00 ^a
4g NaCl	37.50±1.70 ^b	42.50±4.00 ^b	6.00±0.20 ^{ab}	36.00±2.00 ^b
Aerated water (control)	26.00±2.20 ^c	74.00±2.00 ^a	0.00±0.00 ^c	70.00±0.00 ^a

WEANING PERIOD FOR *Heterobranchus bidorsalis* LARVAE

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Heterobranchus bidorsalis is the second most important clariid catfish used for aquaculture in Nigeria. The high mortalities of its fingerlings have hindered its production. This is due to lack of knowledge of optimum weaning period for its larvae. The aim of this study therefore was to determine the optimum weaning period that will enhance high growth and survival. Different weaning period were assessed: 1, 5, 10 and 15 days after yolk absorption (W1, W5, W10 and W15). In the control treatment, larvae were fed *Artemia nauplii* throughout the feeding trial (AP). *H. bidorsalis* larvae (3.5mg) were stocked in glass tanks (60 x 40 x 40 cm³) at 30 larvae/tank. Weight of larvae was determined every 5 days and mortalities were recorded daily. All data collected were subjected to one-way analysis of variance (ANOVA) test and least significant difference (LSD) test at $P = 0.05$ significance level.

Growth of *H. bidorsalis* larvae increased proportionally with weaning period up to 10 days after yolk absorption and no significant growth were achieved by weaning above 10 days after yolk absorption. FGR was lower at W10 than W15 and AP. Suggesting that the larvae utilised a large proportion of the nutrients at this weaning period. The differences in the mean body weight gain at different weaning periods could be attributed to the difference in the stomach functioning state. Fish larvae fed on treatment AP grew faster than larvae on treatment W1. This may be due to ability of the larvae to detect their food. As from day 10 onwards, the survivals in larvae weaned on day10, day15 after yolk absorption and the control were similar but the larvae weaned on day1 and day5 after yolk absorption had significantly lower survival. This was probably due to the inability of larvae to ingest artificial diet at early stages of development, because of its non-functional stomach, with a short digestive tract and few functional enzymes. The results of this study show that food requirement of *H. bidorsalis* changes with age. Weaning of *H. bidorsalis* from *Artemia* to artificial diet should be at day10 after yolk absorption to significantly improve larval production.

Table1 Growth performance of *Heterobranchus bidorsalis* weaned at different period.

	Treatment				
	W1	W5	W10	W15	AP (Control)
Initial weight (mg.)	3.5±0.00 ^a	3.5±0.00 ^a	3.5±0.00 ^a	3.5±0.00 ^a	3.5±0.00 ^a
Final weight (mg.)	6.76±2.10 ^a	15.45±2.30 ^b	30.3±2.60 ^c	28.50±2.40 ^c	27.62±2.00 ^c
Weight gain (%) ¹	329.75	1195.65	2680.32	2490.53	2410.90
	±21.00 ^a	±23.00 ^b	±26.00 ^c	±24.00 ^c	±20.00 ^c
Specific growth rate (%/day) ²	2.08±1.11 ^a	4.92±0.50 ^b	7.12±0.39 ^c	6.96±0.28 ^c	6.92±0.18 ^c
Feed gain ratio ³	2.52±0.27 ^a	2.08±0.21 ^b	0.98±0.12 ^c	1.76±0.15 ^c	1.79±0.15 ^c
Survival (%)	22.50±	57.00±	82.00±0.00	79.50±	80.00±

SUSTAINABLE CARP CULTURE THROUGH PARTICIPATORY AQUACULTURE TECHNOLOGY ASSESSMENT AND REFINEMENT BY WOMEN SELF HELP GROUPS

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Carp have been the mainstay of culture practice in India. Carp farming in the country demands sufficient number of stockable carp seed. There is a great scope of carp seed raising in the rural sector to supplement the increasing demand of fish seed as well as to provide income and employment generation. Analysis of resources and needs have lead to the conclusion that women can be attracted for fish seed production and composite fish culture.

Five technologies currently being investigated starting from spawn rearing to induced breeding of common carp involving women self help groups

Table 1 Spawn rearing technology intervention on-farm-trial on eradication of insects and pests from nursery ponds.

Year	Nursery ponds (Avg. 0.02 – 0.06 ha) WSHG's members	Spawn stocking rate (Lakhs/ha)	Treatments Litres/ha	Spawn yield (Lakhs)	% survivability	Net returns (Th. Rs/ha)	B:C ratio*
2001	15 (176)	30	Farmers practice	1.5	15	30	2.0
			Kerosene (60)	2.5	25	50	3.59
			HSD 60	3.0	30	52.5	3.87
2002	18 (220)	30	Kerosene (60) + Detergent (40)	2.8	28	55	3.65
			HSD (60) + Detergent (40)	4.5	45	90	5.02
			Veg. Oil (60) + Detergent (40)	5.5	55	100	5.79
			Veg. Oil (60) + Teepol (40)	6.5	65	111.5	6.25

* Calculated on the prevailing cost of inputs and products in market.

The information on carp seed rearing technology, their assessment and refinement has raised the confidence of complex-diverse-risk prone poor WSHG's and a horizontal expansion of the fish seed rearing women farmers have been observed. The strong traditional knowledge supported by scientific inputs in the form of various interventions were the factors responsible for such overwhelming adoption.

HATCHERY PRODUCTION AND GROW-OUT FARMING OF FRESHWATER PRAWN IN INDIA – RECENT DEVELOPMENT

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Aquaculture is emerging as a thrust area in producing the required aquatic food, which is proteinaceous and rich in highly unsaturated fatty acids (HUFAs) and considered as healthy food. The major group in freshwater aquaculture is carps, the culture of which is not lucrative, since profit margin to aqua- farmers is small. The next important group is freshwater prawns. The giant freshwater prawn, *Macrobrachium rosenbergii* is a commercially important species. It is a fast growing, hardy, disease resistant species and command an export market.

The global production of freshwater prawn is around 65,000 tons coming from 70,000 ha. The contribution from India is about 30,000 tons for 35,000 ha. There are 71 hatcheries operating in the country and producing 1.83 billion seed. However, the produced seed is not sufficient to meet the demand. Most of the hatcheries are not producing the seed up to their installed capacities due to various problems, such as long larval cycle, lack of efficient larval diets, dependence on *Artemia* and subsequent high cost of production, water quality management and disease outbreaks.

There is a tremendous potential for enhancement of freshwater prawn production in the country. The average production is about 1 ton per hectare/crop, which can be enhanced by manipulation of stocking and judicious harvesting. Size variations and Heterogeneous Individual Growth (HIG) is one of the constraints in grow-out systems under monoculture. By employing poly-culture practice with carps, the above problem could be minimised. In high density monoculture, mono-sex culture is being practiced by the progressive aqua-farmers. Mono-sex population is derived by extended nursery rearing and sex grading by skilled workers. Other problems recently noticed in the freshwater prawn grow-out farming is the soft shelling and white muscle occurrence, which are attributed to poor nutrition, water quality, viral contaminations and other management problems.

INTERSPECIFIC AND INTRASPECIFIC VARIATIONS OF *Echinometra* SPECIES IN OKINAWA

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Interspecific and intraspecific morphological and fertilization variations were studied in three congeneric species of *Echinometra* species B (*E. mathei*), C and D (*E. oblonga*) found off the coast of Okinawa, Japan. These species are distinct in adult and larval morphology as well as species-specificity of fertilization. At higher sperm concentrations ($10^{-5} \times 5$) for conspecific-control, all ova become fertilizable but for heterospermic crosses, fertilization rate was low. Ova from all types of *Echinometra* species B failed to fertilize with sperm from *Echinometra* species C and *Echinometra* species D, but alternatively, sperm from *Echinometra* species B easily fertilized eggs from *Echinometra* species C and D (Table 1). At limiting sperm concentrations ($10^{-6} \times 1.7$), both *Echinometra* species C and D were unable to fertilize reciprocally with *Echinometra* species B. However, crossing between *Echinometra* species C and D produced fertilization membrane at higher and limiting sperm concentrations in both directions (Table 2).

On the other hand, intraspecific fertilization of *Echinometra* B showed different pattern of fertilization when the sperm concentrations were limiting. We also observed that *Echinometra* species B showed intraspecific morphological variation in addition to variability in fertilization success. Intraspecific morphological and fertilization variations within the species may be due to a number of genetic factors including bindin alleles/genotype, egg-sperm collision or non-genetic factors such as nutritional condition, habitat, gonadal maturity, gamete conditions or environmental cues. Such intraspecific variation within the species may imply that *Echinometra* species B is now speciating very slowly.

Table 1 Mean percent of fertilization in conspecific and heterospecific crosses at higher sperm concentration in which 100% of the conspecific eggs were fertilized. Each mean value represents 6 replicates crosses.

Male \ Female	<i>E. sp. B</i> (brown)	<i>E. sp. B</i> (green)	<i>E. sp. B</i> (dark green)	<i>E. sp. C</i>	<i>E. sp. D</i>
<i>E. sp. B</i> (brown)	100 ^a	100 ^a	100 ^a	45.8 ± 1.92 ^c	17.25 ± 1.36 ^c
<i>E. sp. B</i> (brown)	100 ^a	100 ^a	100 ^a	30.2 ± 1.30 ^e	13.79 ± 1.01 ^c
<i>E. sp. B</i> (dark green)	100 ^a	100 ^a	100 ^a	36.0 ± 1.58 ^d	18.76 ± 0.98 ^c
<i>E. sp. C</i>	0 ^b	0.80 ± 0.84 ^b	0 ^b	100 ^a	84.4 ± 0.89 ^b
<i>E. sp. D</i>	0 ^b	0.40 ± 0.55 ^b	0 ^b	90.6 ± 1.14 ^b	100 ^a

* Mean values in the same column having the same superscript are not significantly different (Tukey's test, $p > 0.05$)

Table 2 Mean percent of fertilization in conspecific and heterospecific crosses at limiting sperm concentration in which 60% or below of the conspecific eggs were fertilized. Each mean value represents 6 replicates crosses.

Male \ Female	<i>E. sp. B</i> (brown)	<i>E. sp. B</i> (green)	<i>E. sp. B</i> (dark green)	<i>E. sp. C</i>	<i>E. sp. D</i>
<i>E. sp. B</i> (brown)	41.33 ± 2.4 ^b	40.33 ± 9.7 ^b	39.22 ± 7.9 ^b	0 ^c	0 ^c
<i>E. sp. B</i> (brown)	47.11 ± 8.5 ^{ab}	59.78 ± 13.4 ^a	50.11 ± 9.3 ^{ab}	0 ^c	0 ^c
<i>E. sp. B</i> (dark green)	51.22 ± 10.5 ^a	57.44 ± 13.9 ^a	50.44 ± 13.5 ^a	0.44 ± 0.7 ^c	0 ^c
<i>E. sp. C</i>	0 ^c	0 ^c	0 ^c	53.73 ± 11.8 ^a	4.33 ± 1.11 ^b
<i>E. sp. D</i>	0 ^c	0 ^c	0 ^c	7.07 ± 0.86 ^b	67.44 ± 11.1 ^a

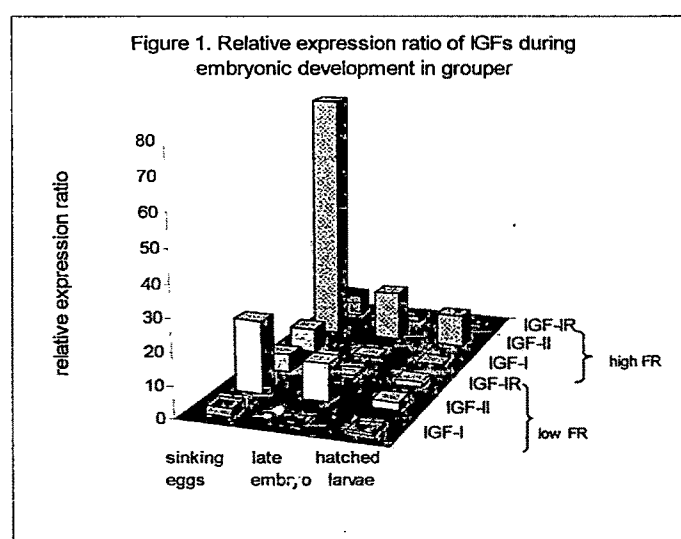
* Mean values in the same column having the same superscript are not significantly different (Tukey's test, $p > 0.05$)

IGF-I, IGF-II AND IGF-IR AS MOLECULAR MARKERS FOR EGG QUALITY

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The common measure of egg quality in fish has been survival to specific developmental stages. Determinants of egg quality are variable among and between teleost species and no common criteria have been established. This study aims to look at expression of growth factors in the developing embryo as potential molecular indicators for egg quality. Insulin-like growth factors (IGFs) are peptide hormones that are important for growth functions and have been shown to induce final oocyte maturation in fish.

Through reverse-transcription polymerase chain reaction (RT-PCR), we have isolated and cloned fragments of the cDNA of IGF-I, IGF-II and IGF-IR in brain, gonad and liver tissues of grouper, *Epinephelus coioides*. From the deduced sequence, primers were designed for use in the quantitative PCR (QPCR) using SybrGreen® as the fluorescent probe. Relative expression ratio of the three genes in sinking eggs, late embryo and newly hatched larvae were calculated using Pfaffl method. Results showed relatively higher expression of the three genes in egg batches with high fertilization rate (FR) than those with low FR in all egg stages examined. IGF-I showed consistent high level of expression related to hatching rate (HR) as compared to IGF-II and IGF-IR. This study also confirmed published findings of decreasing level of IGF expression as development progresses, from the spawned egg to hatched larvae. Although it still needs to be established that these mRNAs are translated into biologically active-proteins, this study has established a direct correlation of IGFs as a marker for egg quality and could serve as a model for other fish species.



IMPACT OF DIETARY *Quillaja saponins* ON GROWTH, SEX RATIO AND REPRODUCTION OF NILE TILAPIA *Oreochromis niloticus* UNDER FIELD CONDITIONS IN BANGLADESH

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Tilapia aquaculture research focuses on environmentally friendly techniques to improve growth performance and to reduce uncontrolled reproduction lately. It was shown in laboratory experiments that a supplementation of the diet with *Quillaja saponins* resulted in increased growth, and suppression of reproduction in adult tilapia and a higher proportion of male fish in tilapia larvae. These findings were tested under field conditions in Bangladesh during a 4-month experiment.

Ten day old tilapia larvae were divided into four feeding groups and their diets supplemented with different doses of *Quillaja saponins* (QS) for five weeks. The treatments were control (no supplementation) and three groups fed diets supplemented with QS at 150 ppm, 500 ppm and 2000 ppm. After 5 weeks the fish were grown-out in hapas in a pond and fed a standard diet without saponins.

At the end of the experiment, the final body weight, weight gain, specific growth rate (SGR) and the assimilation of nutrients in the group supplemented with 150 ppm QS were higher compared to the control, even though the differences were not statistically significant. The SGR and weight gain of tilapia fed diets supplemented with 500 and 2000 ppm QS were similar to the control. Food conversion efficiency was higher in all treated fish compared to the control group. The sex ratios in those fish supplemented with 150 and 500 ppm QS did not differ significantly from 50:50, but the share of males was slightly higher in both treatments compared to the control group. Fish in these groups produced normal fry. Fish whose diets were supplemented with 2000 ppm QS, did not reproduce at all. This could have been caused by a sterility of either males or females. Supplementation of the diet with QS could potentially be used for control of reproduction in tilapia.

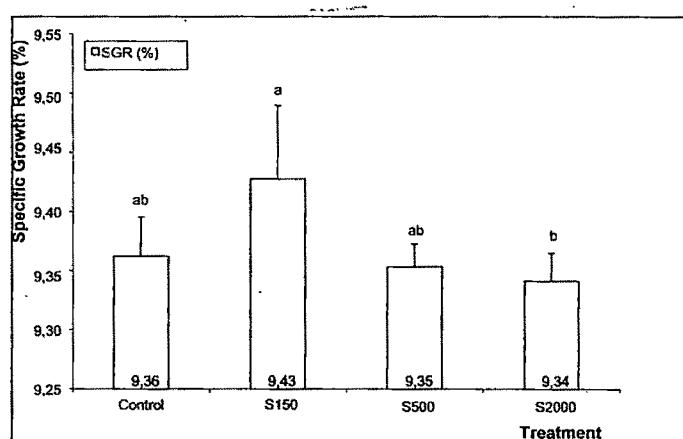


Figure 1 Mean specific growth rate (SGR) of Nile tilapia fed diets supplemented with different levels of *Quillaja saponins*. Bars not sharing the same letter are significantly different ($p < 0.05$).

THE REPRODUCTIVE BIOLOGY OF *Polymesoda erosa* FROM NORTHERN AUSTRALIA

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The reproductive biology of *Polymesoda erosa* (Solander, 1786) (Bivalvia: Corbiculoidae), a staple food for indigenous Australians living in Maningrida Region, northern Australia, was studied between June 2001 and September 2002 as part of a program to manage its fishery and develop hatchery technology for the species. *P. erosa* is dioecious (gonochoristic) which mature at shell length of about 45 mm. Within the range of shell length 55 to 90 mm, the sex ratio-in-population was 1 male:1 female. However, at the shell length lower than 55 mm males were significantly more predominant than females. Females were more common at the shell length larger than 80 mm, but not statistically significant. At the shell length higher than 90 mm, this clam was probably in the senility state when production of new gametes was reduced. Based on the gonadosomatic index (GSI) and histological study, *P. erosa* spawns during the middle until late rainy season *i.e.*, February and May. Males and females showed a synchrony in spawning. Females within the shell range of 65 to 85 mm produced at least 800,000 eggs per animal. The high frequency of spawning was likely related to long inundation during the rainy season. The present practice of not gathering mangrove clams during the rainy season may be important in sustaining the current subsistence harvest. Future research into re-seeding/stock enhancement will constitute the next phase of research prior to further commercial development of this species.

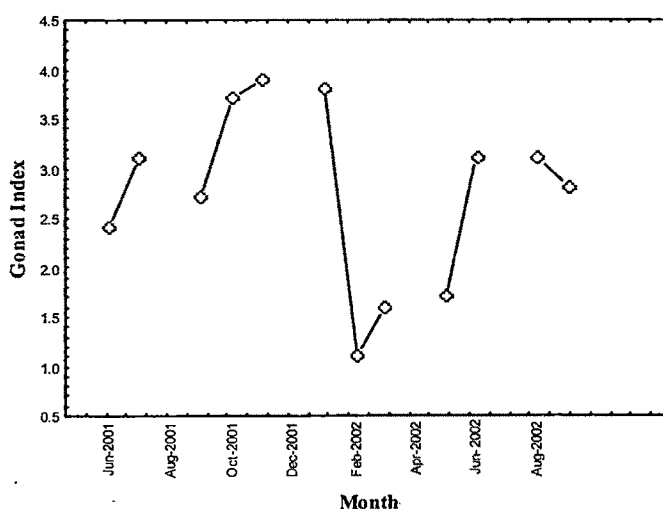


Figure 1:
Seasonal change
in gonad index of
female
Polymesoda erosa
during June 2001
and September
2002.

SEASONAL OVARIAN CHANGES IN THE SPOTTED SNAKEHEAD *Channa punctatus* UNDER CAPTIVITY

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The spotted snakehead *Channa punctatus* is one of the commercially important air breathing fish, and highly regarded in India as a food fish.. An investigation was carried out to determine the annual cyclical changes during the ovarian activity and fecundity of *C. punctatus* under captivity from April 1999 to March 2000. A total of 700 *C. punctatus* ranging in total length from 12-20 cm and weighing 12 -110 g were collected from wild population in a local pond and were stocked in an earthen pond of 3 x 3 x 1m. The fishes were fed with chicken viscera *ad libitum* throughout experimental period. For studying maturation and gonadal development, monthly samples of 4 - 5 female fishes were collected. Altogether 50 females were examined during the period July-December 1999 for fecundity enumeration. Gravimetric method was used for the estimation of fecundity. To establish the relationship between fecundity and total length, and total body weight, gonadal length and gonadal weight, regression coefficients were calculated.

The high gonadosomatic index from April (3.34 ± 0.16) to September (6.15 ± 0.32) clearly indicates vitellogenic period. In May the GSI suddenly increased from 1.17% to 4.42% as the ovaries are in maturing stage, and maximum was observed in September (6.15%) when the ovaries were in ripe condition. The study indicated that July-September is the peak season for the spawning of the fish. The GSI value suddenly declined from 6.15 to 1.19 and the ovary weight was less because most of the ova are shed during this period. The mean ova diameter was maximum (972 μm) during September when the ovaries were in ripe condition and minimum (180 μm) during December when the ovaries were in resting condition. Based on the general appearance of ovary, six morphological stages were classified, viz., I. immature, II. immature, III. maturing, IV. mature, V. ripe and VI. spent respectively. Based on histological observation seven oocytic stages were clearly defined on the basis of nuclear and cytoplasmic characteristics, of which stage I and II are immature, stage III, IV and V are maturing and stage VI and VII are mature ones.

The average fecundity of fish of 110-181 mm in length and 14.4-70.4g in weight varied from 2,116 to 11,332. . The fecundity was found to be linear with respect to body length, body weight and ovary weight. The species is less fecund when compared to Indian carps, catfish and other air breathing fishes. In the present study, the values of GSI and ova diameter increased with the approach of spawning season (July-September) indicating a single spawning period.

**DETERMINATION OF SPAWNING SEASON OF *Garra rufa* (CYPRINIDAE: GARRINAE)
BASED ON THREE INDICES**

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Indices are used in the biological and limnological studies for the easy interpretation of a complex data. For the study of maturation and spawning biology, the Gonado-Somatic Index (GSI) and its modified formula (GSII) or {weight of ovary/weight of fish-weight of ovary}*100 are usually used. Another index is Dobriyal Index (D.I.) which is the third root of gonad weight only. Calculation of GSI and GSII indices involve both the gonad weight as well as the body weight of fish but estimation of D.I. involves only the gonad weight. In this paper, these three indices have been used to determinate spawning season of *Garra rufa* which is known as doctor fish because it is used in the treatment of neurodermitis. During investigation on the biology of this fish, 292 specimens were collected from Zanjiran spring-stream of Fars province, south of Iran. The specimens were fixed in 10% formalin solution, and then were transferred to the laboratory. The fishes were first weighed and then dissected and gonads were removed and finally three indices were calculated. Data showed that 3 indices (GSI, GSII, and D.I.) show almost increasing trend up to April in females when the indices were high as 16.98, 20.45 and 1.27 respectively. There was a sharp fall in the values during May which is indicator of heavy spawning in this month. There was a slight increase during June and slight fall in the month of July which is another attempt for maturation of oocytes and second spawning or late spawning of some fishes. Data revealed that 3 different indices showed similar trend but D.I. index was found more suitable and easy for calculation of sexual maturity and determination of spawning season. As this index involves only the data related to gonads for calculation of index, it is easy for interpretation and calculation and it provides narrow range of index if the gonad weight is very low or very high.

SEASONAL SPAWNING CYCLES OF *Aequipecten opercularis* AND *Pecten maximus* (MOLLUSCA; PECTINIDAE) FROM THE LAXEY BAY AND PORT ST. MARY FISHING GROUNDS IN THE NORTH IRISH SEA, ISLE OF MAN, UNITED KINGDOM

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The scallops of the family Pectinidae, are economically important bivalves in many parts of the world and the reproductive cycles of the species have been studied extensively for the culture practices. The seasonal spawning cycles of Pectinids in Laxey Bay and Port St. Mary fishing grounds have been studied over the period of two years. The objective of this study was to identify the main spawning period in these two locations.

Monthly samples of *Aequipecten opercularis* (queenies) and *Pecten maximus* (scallops) were collected from Laxey Bay of the east coast of Isle of Man at a depth of 20-30m. This was considered to be an inshore area being approximately 6 km from the coast. The second area is about 18.5 km south east of Port St. Mary at a depth of 50-70 m and was considered to be an off shore area. The queenies and scallops were separated and transferred to running seawater tanks with aeration and left for 24 hours. Shell length, shell height and shell thickness were measured and ages were determined. No juvenile animals were investigated so gonad condition was divided into five stages which were termed 'spent', 'recovering', 'filling', 'half-filling', and full'. From the monthly samples approximately 100 individuals of *A. opercularis* and *P. maximus* were taken and their gonad conditions were observed.

Frequency data of the results obtained by three methods of gonads assessment of *A. opercularis* (staging, gonadal index and gonad dry weight) on Laxey Bay it appeared that during May and June there was decrease in full gonad from 51% to 32% suggesting that partial spawning occurred in this period. Rapid recovery was observed in July and August with 60% full gonads by August and in September 95% animals in spent condition indicating major spawning period has taken place. The result observed at Laxey Bay in May which was composed of 33% spent gonads suggests that a substantial spawning had already started in the area. This process continued until June when 72% of the individuals were in the spent condition. The early summer spawning therefore involved a rather higher proportion on Port. St. Mary ground than Laxey Bay.

The main spawning of *P. maximus* in Laxey Bay was in late spring and early summer (79%) (April – June) and there was a small but distinct autumn spawning in August – September (10%). In contrast, the offshore Port St. Mary population only had a single summer spawning (70%) with peak in June – July and there was no evidence of any late summer or autumn spawning.

The study indicated that in both species the main spawning appear to be in early summer and the minor spawning in spring and autumn.

GONADAL CHANGES IN WILD FRESHWATER SPINY EEL *Mastacembelus armatus* IN EARLY SPENT PERIOD

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Histology of the testes and ovaries of wild freshwater spiny eel *Mastacembelus armatus* from Mymensingh was carried out to investigate the gonadal stage during the month of August to November 2003. No male eels were recorded in August and November. In testes, numerous spherical and oval shaped lobule walls were found to be broken during September. Primary germ cells, pycnotic nests of degenerating cells, spermatogonia and spermatids were more and in cluster form during October in comparison with September. In August, ovaries had distinct yolk vesicles stage and yolk granules stage. Oocytes at the yolk vesicle stage tend to lose affinity or stain resembling very faint and appeared empty. During September the follicular cells of the oogonia were ruptured, shrunk and as a result irregular shaped cells were observed. Pycnotic and degenerating nests of oogonia were seen in October surrounded by thecal and follicular cells. Numerous pycnotic cells were also visible during November and broken parts of oogonia were found to be scattered in the area, as a result of which many empty spaces were formed.

OBSERVATION ON THE EMBRYONIC DEVELOPMENT OF BAGRID CATFISH
Pelteobagrus fulvidraco

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The stages of development of laboratory-reared eggs of *Pelteobagrus fulvidraco* are described. The eggs of *Pelteobagrus fulvidraco* are sticky and spherical in shape with a large quantity of yolk and yellow color. The mean diameter of fertilized eggs is 2.03 mm. At the water temperature of 29 ± 0.5 °C, *P. fulvidraco* eggs hatched 33 to 36 hr after fertilization. Embryonic movements of *P. fulvidraco* are described in detail. Some of the eggs break the membrane twice at intervals of several hours and hatchlings are normal.

**GENERATING RESOURCES TO CONTINUE TILAPIA GENETIC IMPROVEMENT R&D
THROUGH PUBLIC-PRIVATE SECTOR PARTNERSHIPS IN THE PHILIPPINES**

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In the 1980s and 1990s, three major tilapia genetic improvement projects were undertaken in the Philippines. These projects were: a) the Fish Genetics Project, b) the Genetic Improvement of Farmed Tilapia (GIFT) Project and c) the Genetic Manipulations for the Improvement of Tilapia (GMT) Project. All these projects were funded by international donor agencies. When grant funding for these projects ended, the institutions involved in the work adopted a number of strategies to generate the funding required to continue the programs.

This paper looks into the various strategies adopted by the institutions with a special focus on the resulting public and private sector partnership models. The paper discusses the successes as well as the shortcomings of the various models that have emerged for the tilapia seedstock industry in the Philippines. A brief review of post-project investments made by the institutions is also provided.

As a result of the Philippine experience in generating resources for the continuation of tilapia genetic improvement research, the paper highlights a number of issues and concerns and makes a number of recommendations regarding public and private sector partnerships.

PERFORMANCE OF STERILE COMMON CARP *Cyprinus carpio* IN POLYCULTURE PONDS

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A technology, to induce sterility in the common carp (*Cyprinus carpio*) has been developed to overcome the problem of unabated breeding in culture ponds. This sterile *C. carpio* grows much faster, yields more meat per unit weight and possess better disease resistance than the normal *C. carpio*. The University of Agricultural Sciences, Bangalore, released this technology for commercial exploitation in 2002.

Farmers have started using sterile *C. carpio* in polyculture as an additional species. They generally stock only catla (*Catla catla*) and rohu (*Labeo rohita*) fingerlings at the rate of 1250 and 8750 respectively per ha and obtain a yield of about 9.5-10 t per year. By introducing sterile *C. carpio* fingerlings into their culture ponds, as a third species, at the rate of about 600-850 fingerlings per ha, they have improved their total production by about 1-1.5 t per annum.

In more than 25 farm ponds, each with a water spread area of about 2 ha, observed by us in about 10 months culture period, the individual sterile *C. carpio* attained a weight of 1.5 to 2.5 Kg. in the districts of Bellary and Raichur in Karnataka, where as the individual weight of *C. catla* and *L. rohita* ranged from 1.0-1.5 kg. The sterile *C. carpio* did not affect the main crop and it formed a good additional crop, thereby increasing the yield and revenue to the farmers by about 10-15%.

The culture of sterile *C. carpio* is steadily gaining popularity among the farmers and consumers. Its use in culture is spreading to other States in India. Three commercial hatcheries have been established in the private sector for the production of sterile *C. carpio* carp seed.

**MOLECULAR DATA FROM THE 16S rRNA FOR THE PHYLOGENY OF CHANNIDAE
Channa sp. IN MALAYSIA**

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Snakeheads are freshwater fish which are distributed widely in Asia and Africa. In Malaysia, six species were identified viz. *Channa lucius*, *C. striata*, *C. micropeltes*, *C. gachua*, *C. maruloides* and *C. melasoma*. In the previous studies, *Channa* species were mainly classified by morphology. Phylogenetic relationships were analyzed using the sequence of 16S rRNA gene. Genomic were extracted from 35 individuals of 6 snakehead species. Primers named forward 16AR (5'-CGC CTG TTT ATC AAA AAC AT-3') and reverse 16BR (5'-CCG GTC TGA ACT CAG ATC ACG T-3') were used for gene amplification. Results indicated that the species belonged two major clades. The first clade belongs to *C. lucius*. The second clade comprises of *C. striata*, *C. micropeltes*, *C. gachua*, *C. maruloides* and *C. melasoma*.

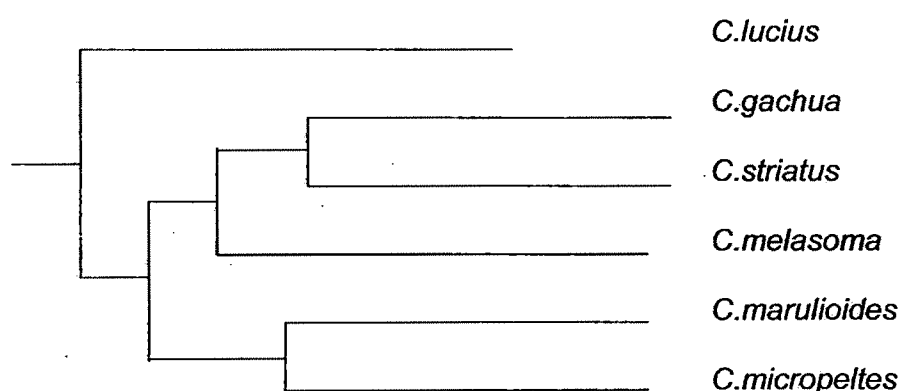


Figure 1 UPGMA phenogram showing the genetic relationship among all individuals of 6 *Channa* species constructed using PHILIP software.

COMPARATIVE STUDY OF GENETIC VARIABILITY BETWEEN RIVER AND HATCHERY STOCKS OF *Catla catla* BY MICROSATELLITE ANALYSIS

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Five microsatellite loci (Cc6, Cc7, Cc8, Cc9, and Ccat A12) were analyzed to study the genetic variation in three rivers: Halda, the Jamuna, and the Padma and one hatchery population of *Catla catla*. The microsatellite alleles were amplified by polymerase chain reaction (PCR), separated on polyacrylamide gel using a sequencing gel electrophoresis system and visualized by silver nitrate staining. Four out of the five loci analyzed (80%) were found to be polymorphic in all the populations. Locus Cc6 had the highest numbers of alleles (6) while the locus Cc9 had the lowest (2). Differences were observed in heterozygosities and average numbers of alleles in the four populations (Table 1) however, no difference was observed in polymorphic loci among the populations. All the studied populations were found to deviate from the Hardy-Weinberg proportions in varied numbers of loci mostly due to the pronounced deficiency in heterozygosity. The hatchery population deviated from river populations in all the four polymorphic loci analyzed. The N_m values and the F_{ST} values indicate a high level of gene flow and lower differentiation between the populations except the Halda population which was more differentiated than the others (Table 2). Nei's genetic distances between the Halda and the other three populations were higher than the genetic distances between all other population pairs. The study revealed a relatively low level of genetic variation in the microsatellite loci within and between the *C. catla* populations and the genetic variation in the hatchery population was lower than any of the three river populations.

Table 1 Mean observed and expected heterozygosity and average number of alleles across five microsatellite loci in four different populations of *Catla catla*

Parameters	Halda	Jamuna	Padma	Hatchery
Observed heterozygosity	0.303	0.291	0.303	0.263
Expected heterozygosity	0.494	0.480	0.502	0.431
Average No. of alleles	4.000	4.200	3.800	3.200
Polymorphism (P95)	0.800	0.800	0.800	0.800

Table 2 *C. catla* multilocus F_{ST} (above diagonal) and N_m (below diagonal) values between pairs of populations across all loci

Populations	Halda	Jamuna	Padma	Hatchery
Halda	****	13.314	12.382	8.868
Jamuna	0.018	****	30.877	91.117
Padma	0.019	0.008	****	16.103
Hatchery	0.027	0.006	0.015	****

CRYOPRESERVATION OF SPERMATOZOA OF INDIAN MAJOR CARPS AND OTHER CULTURE SPECIES IN BANGLADESH

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Cryopreservation of fish spermatozoa is new in Bangladesh. It has immense application in production of genetically good quality fish seed and conservation of biodiversity. Supply of quality fish seed is considered as a pre-requisite for aquaculture development. However, fish seed produced in most of the hatcheries in Bangladesh are integrated with the problems of inbreeding and inter-specific hybridization. These problems can be solved by using cryopreserved sperm from cryogenic gene bank. In order to develop sperm cryopreservation protocols and to standardize them, a series of experiments were carried out with milt collected from Indian major carps (*Labeo rohita*, *Catla catla*, *Cirrhinus cirrhosus*) and also from tilapia (*Oreochromis niloticus*). Different extenders such as modified fish ringer solution (MFR), Kurokura solution, 0.9% NaCl solution and egg-yolk citrate, and different cryoprotectants such as di-methyl sulfoxide (DMSO), glycerol, ethyl alcohol and methyl alcohol were used with different combinations for selecting suitable extenders and cryoprotectants as well as for their suitable combinations. The sperm of Indian major carp cryopreserved with egg-yolk citrate and 10% DMSO exhibited highest (about 90%) post thaw motility followed by 0.9% NaCl solution (about 80%). The Kurokura solution and MFR did not show good sperm motility.

In case of tilapia, sperm cryopreserved with both egg-yolk citrate and MFR mixed with 10% DMSO showed highest post thaw motility (about 80%). The Kurokura and 0.9% NaCl solutions mixed with 10% DMSO did not show good performance. However, about 60-70% post thaw sperm motility was found when egg-yolk citrate was mixed with ethyl alcohol or glycerol. About 40-50% sperm motility was obtained from MFR mixed with ethyl alcohol or glycerol.

COMPARISON OF GROWTH AND REPRODUCTION OF WILD AND HATCHERY PRODUCED STRAINS OF INDIAN MAJOR CARPS

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An experiment was conducted on the comparative growth and reproduction performance between the seed of three species of Indian major carps, rohu (*Labeo rohita*); mrigal (*Cirrihinus mrigala*) and catla (*Catla catla*) from local hatcheries of Jessore and from two natural sources: River adma and River Jamuna of Bangladesh. The growth experiment was conducted for 3.5 months in hapas and 14 months in a communal pond. All the three species of both the riverine stocks grew faster than the hatchery originated stock. The differences obtained between the growths rates of the hatchery and the two rivers were significant at 5% level; the differences between the two rivers were not significant. Rohu and mrigal from the three sources matured in the breeding season of the third year of age and were compared for their readiness for breeding from the early breeding season. Broods from the hatchery source were found be ready for breeding at a higher frequency than either the Padma or Jamuna river sources. Comparisons were also made on the rates of fertilization, hatching and deformity of the developing zygotes. Eggs from the hatchery broods were seen to have significantly lower rates of fertilization and hatching, while having significantly higher rate of deformity in the developing embryos. The spawn produced were again growth tested for comparison between the hatchery and riverine sources in the earthen nursery ponds.

The growth data on weight and length obtained after 18th month of growth showed that the hatchery originated fish were significantly smaller. The SDs of growth in weight was smaller in case of the hatchery fishes than the fishes of the two riverine sources suggesting reduced genetic variability in the hatchery stock. This lower variability in the hatchery population is likely due to the occurrence of inbreeding due to close mating under hatchery conditions. Hatchery fishes, though, were smaller in size than the riverine fishes, became ready for breeding in higher frequency, suggesting the prevalence of negative selection in favor of smaller size broods in the hatchery populations. There is tendency among the hatchery operators for selecting smaller size broods for the reasons of reduced hormone cost and easy of handling of small size broods during spawning in the hatchery. The hatchery population is under inbreeding pressure is further evidenced by the lower rates of fertilization and hatching and higher rate of deformity in the eggs and developing zygotes respectively produced from the hatchery broods. Means for avoiding inbreeding and negative selection in the hatcheries have been suggested in the paper.

ALLOZYME VARIATION OF HATCHERY AND RIVER POPULATIONS OF ROHU *Labeo rohita* IN BANGLADESH

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To assess the population structure of rohu (*Labeo rohita*), five hatchery and three river samples were analyzed by allozyme electrophoresis. Five enzymes specific to *L. rohita* were controlled by eight loci. Two enzymes, EST and GPI, showed diagnostic in both hatchery and river populations, and Mdh-2* locus showed heterozygous for river populations only. Polymorphic loci per population ($41.67 \pm 19.09\%$) and heterozygous loci per individual ($15.48 \pm 2.06\%$) in river populations were higher than those in hatchery populations, ($35.00 \pm 5.59\%$, and $10.67 \pm 3.65\%$, respectively). The heterozygosity ratio (H_o/H_e) was higher in hatchery populations (0.764 ± 0.178) than in river populations (0.581 ± 0.119), probably due to the presence of hybrid individuals in hatchery populations. The high genetic distance ($D=0.263$) between Padma River and other seven populations indicated mix or hybridization between *L. rohita* and other carps species such as *Catla catla*, *Cirrhinus cirrhosus* and *Cyprinus carpio*. The genetically diversified *L. rohita* individuals were observed in Halda and Padma Rivers, while mixed individuals were found in Jamuna River and five hatcheries. The hybridization or complex breeding scenario in hatcheries may have been responsible for this.

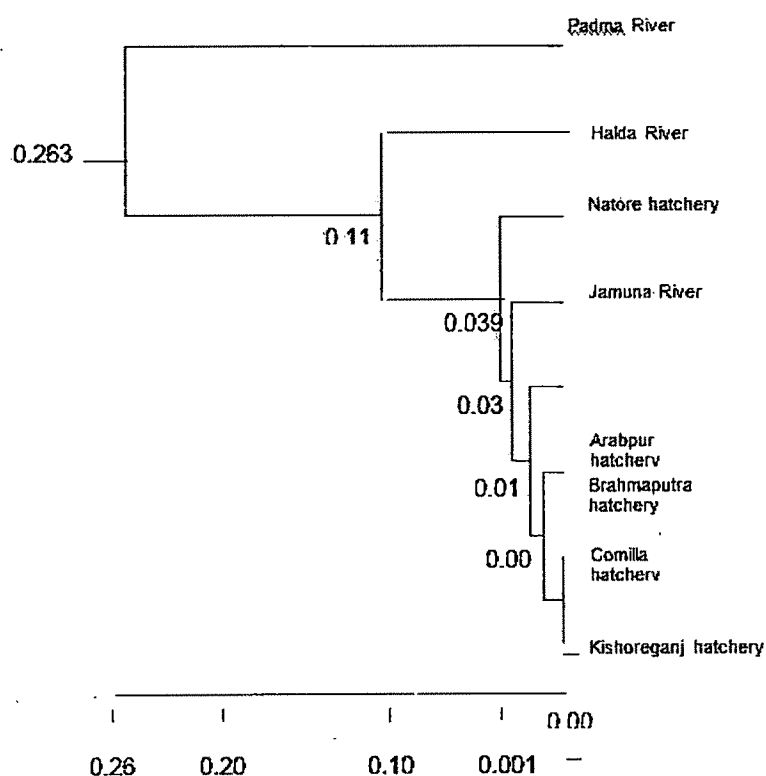


Fig. no. 1. UPGMA dendrogram of *Labeo rohita* populations

GENETIC VARIATION IN THREE RIVER POPULATIONS OF *Cirrhinus cirrhosus*

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Allozyme analysis was conducted for ten enzymes to assess the genetic variation in the Indian Major Carp, Mrigal, *Cirrhinus cirrhosus* from three rivers of Bangladesh: the Halda, the Jamuna and the Padma. A total of ninety individuals, thirty from each population were used for the analysis. Gene products from seventeen presumptive loci were resolved from muscle and liver tissues with three different buffer systems (CA 6.1, CA 7.0 and TC-1). Four loci (*Est-1*, *G3pdh-2*, *Gpi-1* and *Idhp*) were identified as polymorphic ($p \leq 0.95$) while thirteen loci (*Aat-1*, *Aat-2*, *Adh-1*, *Adh-2*, *Est-2*, *G3pdh-1*, *Gpi-2*, *Ldh-1*, *Ldh-2*, *Mdh-1*, *Mdh-2*, *Pgdh* and *Pgm*) were identified as monomorphic. All three populations had the same proportion of polymorphic loci (23.5%). The proportion of heterozygous loci per individual was 16.67% in the Halda and 13.33% in the other two populations. The mean number of alleles per locus was 1.353 ± 0.606 in the Halda, 1.353 ± 0.702 in the Jamuna and 1.294 ± 0.588 in the Padma populations. Average observed and expected heterozygosity were 0.112 ± 0.215 and 0.103 ± 0.195 , 0.124 ± 0.258 and 0.103 ± 0.194 and 0.110 ± 0.229 and 0.086 ± 0.167 for the Halda, the Jamuna and the Padma populations respectively. UPGMA dendrogram (Figure 1) based on Nei's (1972) genetic distance among three river populations revealed that the Halda and the Jamuna populations are genetically closer compared to the Padma population.

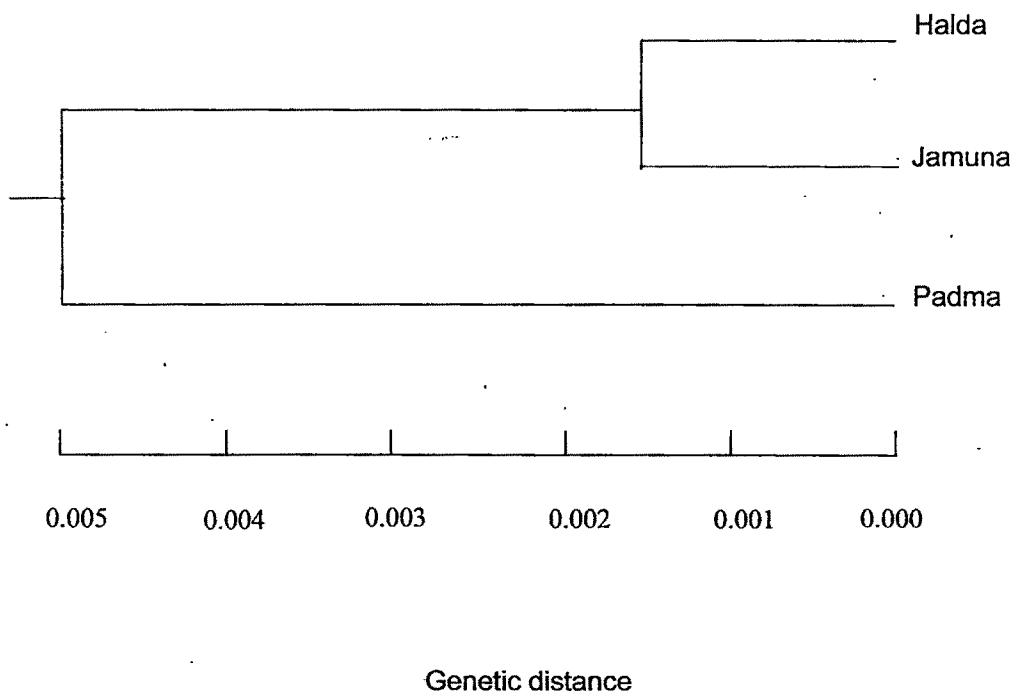


Figure 1. UPGMA dendrogram, showing the genetic relationship among three river populations of mrigal (*Cirrhinus cirrhosus*).

NETWORKING IN RESEARCH: SUCCESS STORY OF INTERNATIONAL NETWORK ON GENETICS IN AQUACULTURE (INGA)

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The importance of genetics and breeding in raising productivity of crops and livestock and their contributions to food security has been well documented. While success has been achieved on these commodities, and recently on temperate finfish, no efforts were made until the 1980s for the genetic improvement of tropical finfish, which contribute about 90% of global aquaculture production. Realizing the need and opportunities for genetic improvement of tropical finfish as demonstrated by the project for genetic enhancement of Nile tilapia (GIFT) and the pressing need for concerted efforts aimed at improving aquaculture productions and conservation of aquatic biodiversity, the International Network on Genetics in Aquaculture (INGA) was established in 1993. INGA is a research network committed to the improvement of aquaculture production through genetics and breeding of cultured species and has membership of developing countries from Asia, Africa, and Pacific, advanced scientific institutions, regional/international organizations and private sector.

Within a decade since its inception, the network has resulted in: (i) development of national breeding programs for genetic improvement of aquaculture species in 13 countries in Asia, Pacific and Africa; (ii) improved breeds of tilapia and carps in member countries (iii) transfer of germplasm among member countries for research and development; (iv) improved capacity in developing country institutions for undertaking fish genetics research; (v) formulation of policy recommendations on issues related to conservation and equitable distribution of benefits from genetic improvement; and (vi) enhanced synergy between genetics research being undertaken at advanced scientific institutions and developing country institutions.

Effective dissemination of improved fish and other products of genetic improvement research in developing countries to benefit the poor farmers remains the major challenge. Thus, the network's next phase will focus on socioeconomic aspects of technology dissemination, including modalities and institutional arrangements needed for making the improved germplasm accessible to rural poor. Through development of appropriate policies/institutional set-up, it is expected that the network will have the following long-term benefits: (i) increased production of fish through aquaculture in developing countries to meet the demand for fish and improve the nutritional status; (ii) equitable distribution of benefits from genetics research to poor; (iii) improved nutritional security of poor producers and consumers in developing countries; and (iv) better conservation of aquatic biodiversity.

The network has made significant strides toward improving productions from aquaculture operations and conservation of aquatic genetic resources and biodiversity. These accomplishments are due largely to close cooperation and complementarities of strengths of all the members participating in the network.

CURRENT STATUS OF GENETICS IN AQUACULTURE IN IRAN

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Regarding the vital role of genetics in aquaculture and fisheries management, the application of genetic techniques should be considered as one of the major tools towards the development of aquaculture industry of Iran.

This paper discusses the status of genetics in aquaculture in Iran. This is with reference to documentation of genetic studies. Also discussed are some of the problems confronting wider application of genetics in aquaculture. This paper reviews the background, the role of research organizations and the future prospects of genetic studies in Iran.

ESTIMATION OF CERTAIN GENETIC PARAMETERS ON GOLDEN Mahseer *Tor putitora* (HAM.)

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The genetic parameters were estimated for Golden Mahseer *Tor putitora* (Ham.) using data from 12 families produced in a nested breeding model and reared for 17 weeks. The average performance of the progeny from each genetic group for growth at different time intervals was studied and comparison made with natural population. They were found to be significantly different. The growth performance was superior in case of natural population. The female F2 performed significantly better than other females and the genetic group M4XF2 was superior in growth compared to other families. Since *T. putitora* is a slow growing fish, the average performance of the families ranged between $21.11\text{mm} \pm 0.21$ to $23.05\text{mm} \pm 0.18$ for length and $154.55\text{ mg} \pm 3.82$ to $167.33\text{mg} \pm 0.93$ for weight at 17 weeks.

Heritability, genetic correlations and phenotypic correlations were estimated from the data. The heritability (sire component) for length ranged from 0.06 ± 0.06 to 0.33 ± 0.20 (week 1 to week 17) and heritability (dam component) ranged from 0.57 ± 0.42 to 0.71 ± 0.51 (week 1 to week 17) respectively. The maternal effects were found to be diminishing after 9 weeks. The estimates of heritability for length were found to be higher than weight. The genetic correlations between the traits ranged from 1.14 to 1.00 from (weeks 11 to weeks 17) indicating a strong relationship. The estimates of phenotypic correlations varied between 0.48 ± 0.06 to 0.25 ± 0.09 from 11 to 17 weeks.

GENETIC IMPROVEMENT STUDIES IN COMMON CARP *Cyprinus carpio*

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Common carp (*Cyprinus carpio*), an introduced species to India, has become an integral part of inland fish culture and enhanced fisheries in Karnataka state. It currently represents 43% of seed stocked and 35% of the state's inland fish production. However, the local strain of *C. carpio* first introduced in the 1950's exhibits precocious sexual maturation and unwanted reproduction suppressing growth and decreasing the yield of marketable fish

To address this problem, a research programme was initiated in the University of Agricultural Sciences, Bangalore. The main objective of the project was to assess the present genetic status of existing stocks of *C. carpio* with a view to enhancing the livelihoods of rural farmers practicing fish culture. Different approaches such as 1) induction of sterility, 2) production of mono sex population and 3) evaluation of different stocks for developing a breeding programme to evolve an improved stock with fast growth rate and late maturity were investigated.

Triploidy was successfully induced using heat shock (40°C for 1.5 min starting 1-3 min post fertilization). The culture performance of triploid fish was evaluated in polyculture and monoculture systems in comparison with diploids. Growth trials indicated that the growth rate of 3n fish was slightly slower than 2n up to the age of sexual maturation (2n/3n=8.5%) but the triploids had better dress out proportion compared to diploids (2n/3n=14.9%).

Indirect production of mono sex fish through combining hormonal sex reversal and progeny testing was evaluated. A number of androgens including 17 α -methyl testosterone (MT), methyl di-hydroxy testosterone (MDHT) and androstenedione (AN) were evaluated using fry of different size and age group. MDHT treatments at 50 or 100 mg.kg⁻¹ feed for 40 days induced complete masculinization when initiated on 50 day old fish. These results are discussed in context of developing a breeding programme for the production of all female *C. carpio* which have superior culture performance compared to mixed sex stocks.

As another option, different stocks of *C. carpio* introduced from Hungary (Amur and P3), Indonesia (RJ) and Vietnam (SV), alongside two local stocks (L-BRP and L-FRSH) were evaluated in a series of culture performance trials. For harvest weight, the most important trait identified in a series of parallel studies with farmers, the stocks ranked Amur > P3 > RJ > L-BRP > SV > L-FRS. Heterosis was negatively correlated with mid-parent values for both harvest weight and RAPD variation ($P < 0.01$ in both cases), and the best performing crosses were not superior to the best performing pure stocks.

The above approaches are discussed in context of providing a viable long term genetic management strategies for the improved performance of *C. carpio* in Indian aquaculture.

SELECTION AND INTRASPECIFIC HYBRIDS IN RED SEA BREAM *Pagrus major* PART I: EFFECTS ON GROWTH PERFORMANCE

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Red sea bream, *Pagrus major*, is an important aquaculture species both in Korea and Japan and many genetic studies of this species were carried out for aquaculture purpose. In this paper the effects of selection by fish size on growth enhancement of Korean strain of red sea bream and the comparative growth performance of selected Korean strain and cultured Japanese strain and their reciprocal intraspecific hybrids are reported.

Offspring from selected line (5th generation of selection) showed significantly better performance in body weight, body length, weight gain, specific growth rate and feed consumption (but not in feed conversion ratio) than offspring from non-selected line (2nd generation of wild) in sea cages rearing trials with communal stocking. At 36 months of age offspring from selected line grew 1.49 times faster in body weight than offspring from non-selected line.

Growth of selected Korean, cultured Japanese and their reciprocal intraspecific hybrids were studied. One month after hatching, two offspring groups from mating with KORDI-F4 females (KORDI-F4♀XKORDI-F4♂ and KORDI♀XJPN♂) showed significantly larger in length than offspring groups from mating with JPN females (JPN♀XKORDI-F4♂ and JPN♀XJPN♂) presumably due to the small egg size of JPN females. In sea cages, offspring groups from JPN♀XKORDI-F4♂ showed significantly faster growth ($P<0.05$, ANOVA, $n=240$) than other offspring groups.

These results demonstrate that the selection effects on growth performance in Korean strain. 1 Intraspecific hybrids between cultured Japanese female and selected Korean male showed the heterosis in growth and resistance to low water temperature.

**PART II: FEED UTILIZATION OF OFFSPRING FROM SELECTED KOREAN STRAIN,
CULTURED JAPANESE STRAIN AND THEIR INTRASPECIFIC HYBRID OF RED SEA
BREAM, *Pagrus major***

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Feeding and starvation experiments were conducted to investigate the differences in growth performance, total ammonia nitrogen (TAN) excretion rates and feces production rates among the offspring from selected Korean strain (KK, mean body weight (BW); 21.5 ± 0.1 g), cultured Japanese strain (JJ, mean BW; 17.1 ± 0.1 g) and their intraspecific hybrid (JK, mean BW; 17.1 ± 0.1 g) of red sea bream in order to compare their feed utilization efficiency. Fish were hand-fed commercial diet containing 46.7% protein for 2 weeks, three times daily at 09:00, 13:00 and 17:00 hrs. Feeding rate for the fish was 3% body weight per day.

Feed conversion ratio and protein efficiency ratio of KK is superior to JJ and JK ($P < 0.05$). After daily feeding, the TAN excretion rates reached peaks of 49.03, 58.75 and 36.26 mg/kg fish/hr for the JJ, JK and KK strain, respectively, during the daytime. The peak TAN excretion rate of the KK strain was significantly lower than that of the JJ and JK strain ($P < 0.05$), but daily TAN excretion rates of the JJ, JK and KK strain were not different ($P > 0.05$). When fish were fed at satiation after 4-day starvation, TAN excretion rates reached the maximum values 4 hours after feeding for the KK strain (31.23 mg/kg fish/hr) and 6 hours after feeding for the JJ (44.19 mg/kg fish/hr) and JK strain (41.70 mg/kg fish/hr). After 3-day fasting, the daily endogenous TAN excretion rates (ETE) for the JJ, JK and KK strain was 286.91, 215.66 and 179.29 mg/kg fish/day, respectively, the value of the KK strain was lower than that of the JJ and JK strain ($P < 0.05$). The total feces production rates of the JJ, JK and KK strain were not different significantly, but the proportion of feces production rates with time series for the JJ, JK and KK strain were different ($P < 0.05$). Overall, selected strain had higher protein utilization efficiency than non-selected strain and the their intraspecific hybrid.

GENETIC ASSESSMENT AND MANAGEMENT OF CHINESE CARP STOCKS IN BANGLADESH

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Early introductions of Chinese carps into Bangladesh were secondary or tertiary transfers from china, involving intermediate locations, and probably bottlenecking, founder effects, etc. Anecdotal evidence suggested fairly widespread hybridization between silver and bighead carps in Bangladesh, probably resulting in introgression in hatchery broodstock. In 1994, new stocks of silver carp, bighead carp and grass carp were introduced directly from the Yangtze River to the northwest fisheries extension project (NFEP), parbatipur, Bangladesh. This presentation describes a series of studies relating to the evaluation, management and dissemination of these stocks. A broodstock management plan was designed and initiated with the objective of minimising inbreeding in the core broodstock. A series of trials demonstrated that the performance of the parbatipur stocks in polyculture ponds was consistently superior to that of several other stocks. Microsatellite dna loci were isolated from a silver carp DNA library and used to assess intraspecific variation. Three of these loci also consistently differentiated between silver and bighead carp, and were used to assess the level of hybridization in hatchery broodstocks across the country. A range of levels of hybridization was detected in commercial hatcheries (overall frequency 8.3% in silver carp and 23.3% in bighead carp), while no hybridization was detected in the parbatipur stocks. On the basis of these results, widespread dissemination of the parbatipur stocks to the aquaculture industry, via a series of multiplier stations, has been recommended, along with appropriate monitoring of and training in genetic management of broodstock.

MUD CRAB *Scylla serrata* FATTENING IN BAMBOO CAGES IN BANGLADESH COASTAL WATERS

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Present traditional system of mud crab (*Scylla serrata*) fattening in earthen ponds has not been practical and was not successful due to high mortality and low growth rate. This study shows comparison between cage culture and pond culture of mud crab. The higher growth rate (1.12g/crab/day) and less mortality (3-7%) of fattened mud crab were recorded in the cell cage as compared with the earthen pond culture system (growth 0.51g/crab/day and mortality 8-12%). Male crabs had higher growth as compared to females. Mud crab fattening in the cell type of bamboo cages required low investment (Tk.670/crop/cage) as compared to earthen pond (Tk.2350/crop/pond), and was more profitable (about Tk.350 for cell cage, Tk. 224 for pond). The growth and mortality rate of mud crab was influenced by seasons. These two biological parameters were probably related with salinity, temperature and other ecological parameters in the present study area. The present results indicate cell type of cage culture system is more sustainable and economically viable for the poor and landless coastal people.

Table 1 Culture of *Scylla serrata* in the Mathamuhuri river confluence at Chakaria Sundarban area of Bangladesh

Stocking time	Stocking number		Stocking size (gm)		Rearing period (days)	Mean growth $\pm$ SD in total rearing period		Percentage of mortality (%)	
	Male	Female	Male	Female		Male	Female	Male	Female
12.06.03	30	30	160-181	140-164	15	24.66 $\pm$ 5.99	16.00 $\pm$ 4.68	3.33	0.00
26.07.03	30	30	300-348	200-250	14	21.03 $\pm$ 6.55	8.51 $\pm$ 4.78	3.33	0.00
06.10.03	30	30	250-400	170-235	15	20.52 $\pm$ 4.72	15.03 $\pm$ 4.04	3.33	3.33
09.11.03	30	30	250-395	170-250	15	14.14 $\pm$ 5.85	13.79 $\pm$ 4.51	3.33	3.33
24.12.03	60	60	250-300	178-247	15	18.21 $\pm$ 5.87	14.98 $\pm$ 4.80	3.33	-
06.03.04	30	30	250-300	180-230	14	12.82 $\pm$ 6.78	10.71 $\pm$ 6.19	6.66	6.66

COMMUNITY BASED MOLLUSC CULTURE IN BANGLADESH COAST: AN ALTERNATIVE INCOME GENERATING OPTIONS FOR THE POOR

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Unique physical and biological conditions at Cox's Bazar coast, southeastern Bangladesh, are responsible for an accumulation of high amounts of mollusc spat. Direct settlement of the spat to suspended cultch in the water column was compared by using locally available selected materials (e.g. plastic sheet, pottery, windowpane shell and bamboo) at Gorakghata of Moheshkhali channel and Chufaldhandi canal of Cox's Bazar coast. The prepared cultch frames were installed at different depths of two selected sites for spat collection. Bamboos of various sizes were used for raft construction. Plastic drums of 200 l, one on each corner, were used as floats to keep the raft afloat. Iron anchor was attached to the raft. Cultch, rope and tray were hung from the raft for culture in both sites. Material costs for windowpane shell, pottery, bamboo (10 poles), and plastic sheet cultch were US\$ 0.29, 0.98, 0.95 and 1.24, respectively where average spat density was found 0.34, 0.16, 0.02 and 0.14/cm² of oyster and 0.69, 0.33, 0.03 and 0.27/cm² of green mussel in the said cultch. Cultch-wise monthly average growth rates were measured as 0.6-0.9, 0.6-0.8, 0.5-0.9 and 0.5-0.9 cm for oyster, and 0.4-0.9, 0.4-0.7, 0.3-0.6 and 0.3-0.9 cm for green mussel. The economic analysis, spat density and monthly average growth rate indicate that windowpane shell is the most viable spat settling material for the coastal poor communities. Total materials costs for 1 raft with preparation and installation were US\$ 23.53 where total production was 115 kg oyster and 92 kg green mussel. Muscle weight was 18 kg oyster (16%) and 38 kg green mussel (41%) with selling price of US\$ 1.72 and 1.03 respectively in Rakhain community. Shell was sold to the lime producers and poultry feed mills at the rate of US\$ 0.086/kg for oyster and US\$ 0.14/kg for green mussel. Total selling price of meat and shell was US\$ 86.16 and the profit was US\$ 62.62 in 8 months from 1 raft.

Table 1 Cost, monthly spat density and growth rate in different types of cultch (Although data have been collected from Oct 2003, only Jan-May 2004 data are presented here)

Cultch types			Windowpane shell		Pottery		Plastic sheet		Bamboo	
Cost for 1 cultch frame (US\$)			0.29		0.98		1.24		0.95 (10 poles)	
Cultured species			Oyster	Mussel	Oyster	Mussel	Oyster	Mussel	Oyster	Mussel
Months	Jan. 2004	Total spat	6	13	12	21	6	14	7	19
		Spat/cm ²	0.08	0.17	0.05	0.10	0.03	0.07	0.005	0.01
		Spat size (cm)	2.3	1.7	2.1	1.7	2.0	1.6	1.8	1.6
	Feb. 2004	Total spat	10	18	14	29	9	17	6	11
		Spat/cm ²	0.13	0.23	0.06	0.13	0.04	0.08	0.006	0.007
		Spat size (cm)	2.9	2.1	2.8	2.3	2.6	2.2	2.7	2.1
	Mar. 2004	Total spat	21	38	27	47	25	42	14	22
		Spat/cm ²	0.27	0.48	0.11	0.21	0.12	0.20	0.01	0.02
		Spat size (cm)	3.8	2.9	3.5	2.8	3.2	2.7	3.6	2.7
	Apr. 2004	Total spat	23	51	33	69	31	58	19	37
		Spat/cm ²	0.30	0.65	0.15	0.30	0.15	0.28	0.01	0.03
		Spat size (cm)	4.7	3.3	4.2	3.1	4.1	3.0	4.4	3.2
	May 2004	Total spat	49	94	51	103	38	81	36	61
		Spat/cm ²	0.62	1.20	0.22	0.45	0.18	0.39	0.03	0.04
		Spat size (cm)	5.6	4.2	5.0	3.5	4.8	3.4	5.2	3.5

STATUS OF CAGE CULTURE IN LAO PDR

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Lao PDR has abundant aquatic resources, including reservoirs, back swamps, the Mekong River and its tributaries. Many locations are suitable for nursing and grow out in cages, and cage culture has increased dramatically in recent years. In 2002 a survey of cage culture was carried out by the Living Aquatic Resources Research Center (LARReC).

There is an increasing demand for fish produced from cage culture, and as a consequence, the demand for seed, feed and other inputs are also expected to increase. There are approx. 2,000 cages of different sizes scattered in 7 provinces. The total feed requirement is estimated to about 2,000 tons/year. The fish seed requirement is more than 2.3 million fingerlings / year. The major part of fish seed and fish feed requirements are currently imported from neighbouring countries. Substantial increases in cage culture development will require improved and increased seed and feed production.

Cage culture in Lao PDR may be divided into three categories: (1) Tilapia and some other carps, (2) Carnivorous fish species, e.g. *Channa striata* and *Channa micropeltes*, (3) Silver carp and big head carp. Although tilapia is the main species for cage culture, an increasing proportion is indigenous species. The average FCR for the first and second group is 1.6 and 3.5 respectively. The third group utilizes natural food, and additional feeding is not required.

It is suggested to support an increase in Indigenous fish species in cage culture. Further, the country needs to become self-sufficient in the production of good quality fish seed and increasing the amount of locally produced feed by using all local available raw materials.

A BIOECONOMIC EVALUATION OF TILAPIA MONOCULTURE AND POLYCULTURE WITH FRESHWATER PRAWN WITH AND WITHOUT PERIPHYTON SUBSTRATES

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The effects of periphyton, grown on bamboo substrate, on growth and production of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) and giant freshwater prawn (*Macrobrachium rosenbergii*) in monoculture and polyculture systems were studied at the Bangladesh Agricultural University, Mymensingh. The experiment had 2x2 factorial design: monoculture of GIFT at 20,000 fingerlings.ha⁻¹, with and without substrates and polyculture of GIFT at 20,000 fingerlings.ha⁻¹ and prawn at 20,000 postlarvae.ha⁻¹, with and without substrates. Twelve ponds (area 75m² and depth 1.5 m) were assigned randomly to the four treatments in triplicate. Bamboo poles (mean diameter 5.5cm and 5 poles.m⁻²) were posted vertically in ponds under substrate treatments. There were statistically significant differences in secchi disc depth, pH and phytoplankton and zooplankton abundance and community composition among treatments. A total of 43 genera of phytoplankton and 17 genera of zooplankton were identified from the pond water whereas, 41 genera of phytoplankton and 6 genera of zooplankton were identified from the periphyton samples. Survival of GIFT and prawn was higher in ponds with bamboo substrate than in the control. Addition of substrate and prawn into GIFT ponds significantly increased growth and production of both experimental species. In monoculture, substrate contributed to GIFT production by 39%, whereas, in polyculture, it contributed to GIFT production by 49% and to prawn production by 127%. Highest total yield over 130 days culture period was recorded in substrate-based polyculture ponds contributing a net profit margin of BDT 64,000.ha⁻¹ as compared to other treatments (Table: 1).

Table 1 Production (± SD) and income from ponds with and without substrate over 130 Days.

		Without substrate		With substrate	
		Monoculture	Polyculture	Monoculture	Polyculture
Production (kg ha ⁻¹)	GIFT	1,712 ± 20	1,691 ± 113	2,376 ± 240	2,523 ± 168
	Prawn	--	62 ± 9	--	141 ± 10
Economics	Expenditure	91,000	106,000	114,375	129,375
(*BDT ha ⁻¹)	Gross income	102,695	120,166	142,589	193,798
	Net income	11,695	14,166	28,214	64,423

*1 US\$ = 58 BDT

This study suggests that addition of high valued prawn to periphyton-based tilapia ponds resulted in higher fish production and better economic returns which might be a promising option for low-input ecological aquaculture systems.

PERFORMANCE OF MUD CRAB *Scylla olivacea* CULTURE IN BAMBOO PENS AT DIFFERENT STOCKING DENSITIES FROM SOUTHWEST BANGLADESH

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The feasibility of female mud crab (*Scylla olivacea*) cultured in 100 m² bamboo pens was assessed in terms of growth, survival, production, FCR and ROI, through three stocking densities (SD) (0.25, 0.5 and 1.0 crab/m²) with three replicates for each SD. The study was conducted for a period of 60 days at Munshiganj of Satkhira district (22°12'19.7" N; 89°09'1.8" E), Bangladesh from October to December, 2003. The average body weights of crabs at stocking were 102.69±1.66, 120.27±1.93 and 141.69±2.38g for SD 1, 0.5 and 0.25 crab/m² respectively. The crabs were fed with low cost trash fish (Tk. 20/kg) at 5% of their biomass per day in the morning and evening. The water height in the pens were maintained between 0.7 and 1 m. Total water of the pens was changed during spring tide phases fortnightly.

The average water temperature, salinity, pH and DO were recorded at 30 °C, 3 ppt, 7.7 and 6.2 ppm respectively. After 60 days, stocked crabs attained an average size at 147.33±0.58, 183±1 and 193±2 g for SD 1, 0.5 and 0.25 crab/m² respectively. The best average growth based on length and weight (7.79±1.04 mm and 62.73±2.19 g) were attained at SD 0.5 crab/m² followed by 0.25 and 1 crab/m². The highest survival (79.33±1.53%) was found at SD 0.25 followed by 0.5 and 1 crab/m². The highest production (8.29±0.14kg/100m²) was achieved at SD 1 followed by 0.5 and 0.25 crab/m². The lowest FCR (1.69±0.12) was found for SD 0.5 followed by 0.25 and 1 crab/m². The best return on investment (172.91%) was found for SD 0.5 followed by 0.25 and 1 crab/m².

A negative but significant correlation was found for growth rate and survival with stocking density while production and FCR showed significant positive correlation with SD. However, ROI showed negative and insignificant relationship with SD. The survival and production were found to be better with SD 0.25 and 1 respectively while comparatively better performance of growth, FCR and ROI were achieved with SD 0.5 over the 0.25 or 1 crab/m². Therefore, a stocking density of 0.5 crab/m² could be most suitable for commercial farming practice of mud crab through bamboo pen system in Bangladesh.

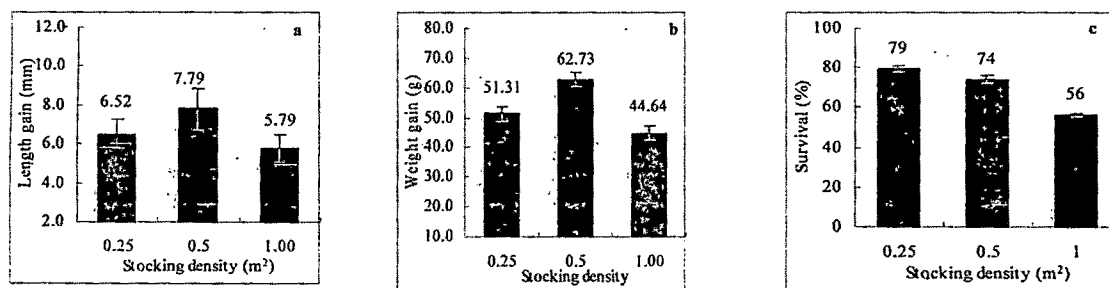


Fig. 1 Effect of stocking densities of mud crab culture in bamboo pens- a: length; b: weight gain; c: survival.

AQUACULTURE IN LANDLOCKED STATES OF INDIA-AN APPRAISAL, CONSTRAINTS AND DEVELOPMENT STRATEGIES

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India with its varied agro-climatic zones is endowed with rich and varietal aquatic resources from temperate waters of North to subtropical and tropical waters of down south. Landlocked states are contributing 16.86% of the total fish catch and almost 1/3rd of the Inland fish production of the country.

The scenario of aquaculture in landlocked states especially the North-West plains of India is different from that of coastal states. In view of diversification from agriculture (being agricultural states), some farmers have converted part of their agriculture land into fish farming units, and others have taken village ponds on lease. Carps are generally cultured in these states by adopting semi-intensive polyculture system, which fetch low price in the local markets as people do not like to consume carps due to the presence of intramuscular bones. This necessitates promoting the culture of other economically important fish which have less bones and high market price. Unlike coastal states, temperature fluctuations are quite high in these states and the growth of carps is quite less during winter. Further in view of carp diversification, only one crop (during summer) of freshwater prawn can be taken. So there is need to introduce those species which can grow well even at low temperatures. Further, the states of Haryana, Punjab, Rajasthan and Western Uttar Pradesh, have more than 3.2 million ha salt affected land, where, in some pockets (in different depths) salinity is even more than 30ppt. These lands are unsuitable both for agriculture and carp culture. In areas, where the level of salinity is alarmingly high, the culture of brackish water fishes can be promoted and in low saline zones (up to 7.00 ppt) carps and freshwater prawn can be cultured. Further in high saline zones water salinity can be decreased by mixing canal water. These states have bountiful of freshwater resources in the form of reservoirs, lakes, rivers etc.(where fish production is very less) and introduction of cage culture can promote culture based capture fishery in these large water bodies

Further, there is need to i) enhance carp production from existing ponds by adopting intensive culture technologies suited for the region; ii) promote fish based integrated farming, especially for agricultural farmers who are looking for diversification; iii) popularize polyculture of carps with freshwater prawn, which is an exportable commodity with an overgrowing demand; iv) promote export oriented aquaculture industry and production of high value fresh water fishes; v) promote the production of value added fish/prawn products, as these are in good demand not only in local but also in international markets; vi) install fish processing units; vii) promote ornamental fish culture, not only to earn a living but also to earn profits by exporting.

The paper highlights the strategies to formulate detailed integrated plans to promote sustainable aquaculture in landlocked states of India, where, the farmers are highly receptive to adopting modern technologies and willing to invest.

THE POTENTIAL OF SELF- RECRUITING SPECIES (SRS) IN AQUACULTURE FOR SUSTAINING THE LIVELIHOODS OF RURAL POOR IN BANGLADESH

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Self-recruiting species of aquatic animals (SRS) appear to be an important component of the harvest from small-scale, household-managed systems in Bangladesh. Previous studies indicated that they are a particularly important resource for poorer people in environmentally vulnerable areas. There is a widespread perception however, that such species have a negative impact on stocked carp polycultures.

The impacts of incorporating selected SRS into conventional carp polycultures was studied in farmer managed ponds in a drought-prone area of northwest Bangladesh for 12 months. On the basis of farmers' attitudes, interest and resource type three categories of SRS management at the household level were identified as SRS positive (POS), SRS negative (NEG) and SRS neutral (NEU). A total of 30 households from 3 villages were wealth ranked as poor (10 farmer), medium (15) and better-off (5). SRS positive farmers deliberately stocked *Clarius batrachus* and *Heteropneustes fossilis* broodfish and fingerlings of *Anabas testudineus*, *H. fossilis*, *C. batrachus* along with commonly stocked carps while also encouraged introduction of selected wild fish from adjacent aquatic systems where possible. NEU households did not deliberately stock SRS nor prevent their entry or eradicate them once in the system in contrast to NEG households. Household attitudes and activities regarding their managed aquatic systems were monitored monthly. System input/output and consumption data was recorded weekly. Total production was estimated after draining of ponds and final harvest. Estimates of consumption and sale were assessed from all farmers at the end of season.

The production of SRS was significantly greater in POS and NEU than NEG. SRS production in POS households was 2.14 times higher than NEG and 1.52 times higher than NEU. Total harvested yields did not vary with treatment (production of carp and SRS combined); for POS, NEG and NEU yields were 2472.47kg/ha, 1788.28 kg/ha and 2230.41kg/ha respectively. Household category also did not affect the yield of stocked carps ($P>0.05$).

Total consumption of SRS per household in POS was higher than NEG and NEU households ($P<0.05$). The study also revealed that both better-off and poorer farmers tended to consume more SRS than they sold. A minority of poorer farmers (30%) sold more SRS than they consumed however revealing the importance of SRS for income generation among this group.

Managing culture systems to maintain the productivity of SRS may demand only the avoidance of negative actions. The lack of negative impacts on carp yields, and increased production and consumption of SRS from POS/NEU households, confirms their value within culture systems has been greatly underestimated.

EFFECT OF ADDED SUBSTRATES ON THE PRODUCTION AND POPULATION CHARACTERISTICS OF *Macrobrachium rosenbergii*

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The effect of two different types of added substrates on the growth and population characteristics of freshwater prawn *Macrobrachium rosenbergii* raised in polders of Kuttanad (S. India) was evaluated. Additional shelters were introduced either in the form of earthen pipes or tiers of nylon nettings in order to increase the surface area of the polders by 30% and their performance was compared against control ponds in which no substrates were added. Addition of artificial substrate produced linear increase in total production without making any compromise on the average weights. The results of the present study showed polders incorporating tiers of nylon nets gave promising results in terms of net yield and percentage of marketable prawn groups when compared to those with earthen pipes or no substrates. The survival rate, average weight of prawns and total production from polders laid with net-tiers were more (58.66%, 58.78 g and 605.56 kg/ha respectively), when compared with polders laid with earthen pipes (48.35%, 52.25 g and 453.11 kg/ha) or those without any additional substrates (26.84%, 38.46 g and 111.62 kg/ha respectively). The percentage of larger blue-clawed morphotype was significantly higher in polders with added shelters either as earthen pipes (51%) or as net-tiers (48%) when compared to those without (39%) any shelters. The results also showed irrespective of the type, substrate inclusion in polders facilitated faster morphotypic transformation and thenceforth the preponderance of terminal morphotypes. Hence it could be reasonably concluded that addition of tiers of nylon nets were found necessary to improve the survival rate, average weight, net production and better marketable yield structure from *M. rosenbergii* farming. Besides, these types of substrates are easily replaceable at the time of harvest and therefore could be easily managed.

SUITABLE SPECIES COMPOSITION IN THE POLYCULTURE OF THAI PANGUS *Pangasius hypophthalmus* WITH CARPS AND PRAWN

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An experiment was conducted to determine the suitable species composition for the polyculture of Thai pangus (*Pangasius hypophthalmus*) with carps and prawn (*Macrobrachium rosenbergii*) using low cost formulated feed. The experiment consisted of three treatments: monoculture of *P.hypophthalmus* at a density of 70 individuals/40 m² (T1); *P. hypophthalmus* in polyculture with catla (*Catla catla*), rohu (*Labeo rohita*) and *Macrobrachium rosenbergii* at 40, 10, 20, 15 (T2) and 40, 20, 10, 15/40m² (T3). The fishes were fed with 20% protein diet consisting of 40% rice bran, 10% fishmeal, 15% wheat bran, 15% wheat flour, 5% mustard oil cake, 11.5% soybean cake, 0.5% vitamin pre-mix, 2% snail shell and 1% salt, at the rate of 10% body weight during the 1st month and then the feeding rate was reduced up to 3% with the increase in body weight. The water quality parameters were monitored fortnightly.

After 7.5 months of culture, *P.hypophthalmus* reached the highest individual weight of 502.77g in monoculture (T1) followed by 383.28g in T2 and 398.25g in T3. The other three species *C.catla*, *L.rohita* and *M.rosenbergii* showed highest weight gain of 242.37g, 290g and 102.82g in T2, T3 and T2 respectively. The food conversion ratio (FCR) in treatment T1, T2 and T3 was estimated to be 2.17, 2.17 and 2.22 respectively. Fish production was highest in T1 with lowest return because of lower market price for *P.hypophthalmus* as compared to carps and prawn. It might be concluded that polyculture of *P.hypophthalmus* is more profitable than the monoculture. The study also showed that *M.rosenbergii* can be cultured with *P.hypophthalmus*. Though *P.hypophthalmus* is a carnivore, it does not feed on prawn or carp fry if sufficient feed is supplied.

**CAGE CULTURE OF RAINBOW TROUT *Oncorhynchus mykiss* IN STAGNANT WATER
IN LAKE AND RESERVOIR**

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The present study was conducted to utilize the empty cages during winter in mid hill lakes after harvesting of carps. In summer, cages are used to grow plankton-feeding carps, however, in winter carps do not show satisfactory growth due to low water temperature and low productivity of plankton. Thus, mostly carp are harvested at the beginning of winter and the cages remain empty for 3-4 months until the next favorable growing season of carp. The present experiment showed that in a cage of 5 m x 5 m x 2 m dimension in lakes and reservoir when stocked at the rate of 5 trout per cubic meter, a total of 25 kg of trout can be produced with a daily growth rate of up to 0.88 g.

The study has shown that trout culture in cages in mid-hill lakes and reservoirs during the winter from November to March when water temperature remain within 13-22°C, can be undertaken. Using this technique marginal farmers can achieve a benefit of about 2735 Nepalese Rupees* per cage in a period of 100 days. Mechanism for trout seed supply to enhance the further adoption of the technology is recommended.

* 1US\$ = 74 Nepalese Rupees

DOMESTICATION OF SAHAR *Tor putitora* AND ITS DEVELOPMENT IN THE HIMALAYAN KINGDOM OF NEPAL

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Sahar (*Tor* spp.) the second largest indigenous riverine fish species after *Bagarius* spp. is a very popular sport fish inhabiting large rivers and lakes/reservoirs in the Himalayan kingdom of Nepal. It is an omnivore but needs higher animal protein content in its diet for better growth. It is a long migratory fish and has high economic value and can survive from 7-38°C though 25-28°C is suitable water temperature for good growth. The breeding technology for *Tor putitora* has been developed in Nepal but the culture technique is yet to be refined. *T. putitora* and *T. tor* are the two species available in Nepal, *T. putitora* being the most common at an altitude of 70-1891 m while *T. tor* is an endangered species found at an altitude of 135-1891 m. The study has shown that *T. putitora* grows better in warm water with slow growth in first year and increasing from second year onwards. Both *T. putitora* and *T. tor* species are very popular having high demand as well as good protein and income source for the fishermen living along the rivers, lakes and reservoirs. It is a partial breeder with breeding response mostly during monsoon season and breeds from March to November.

The population of the species in natural waters is on the decline due to deteriorating environmental conditions as well as high fishing pressure by electro-fishing, poisoning, dynamiting etc. Therefore the development of breeding and culture techniques of *Tor* spp. including feed will lead to positive development in culture industry as well as help to maintain the declining population through stock enhancement in lakes/reservoirs and rivers. It would also help to develop tourism industry and provide livelihood opportunities for the low-income people living in the rural areas. *T. putitora* grew to 29 g within 7 months culture period in first year and less than 100 g in second year at 12-21°C water temperature in Godawari in the mid hill region at an altitude of 1800 m and to 160 g within 12 months in first year and more than 500 g in second year at above 18.5°C water temperature in Tarahara in the terai region at an altitude of 74m indicating that *T. putitora* is suitable for culture in warmer areas in southern part of the country while the mid hill region with lower temperature is more suited for breeding.

EFFECTS OF DIFFERENT CULTURE SYSTEM, DENSITY AND NUTRIENTS ON *Macrobrachium rosenbergii* PRODUCTION IN AN ARTIFICIAL INTEGRATED CULTURE SYSTEM

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One approach to improve aquaculture production is through improvement of basic aquaculture practices such as the technological development and modeling of a new and multiple production system. Nearly all records of farmed prawn are attributed to *Macrobrachium rosenbergii*, which is one of the largest species of the *Macrobrachium* genus and culture of this species is favored due to good survival, taste and rapid growth in inland freshwater bodies. The current research deals with the performance of operation technique on nutrient optimization, stock density and water purification processes in an artificial enclosed system.

Data generated through three studies of freshwater prawn indicated that desirable growth rate of *M. rosenbergii* would be possible from 0.1 to 0.5 EC (mS/cm) of supplemental liquid fertilizer. Evaluation of nutrient composition illustrated that copper (Cu) and nitrogenous compound (NH₃) were the critical elements in the water of rearing tanks of the polyculture system. Studies pointed out that some mortality of *M. rosenbergii* can be related to cannibalistic behavior in the culture system. Space limitation would encourage competition and cannibalism of *M. rosenbergii* especially during the molting process. In order to solve these problems, water of culture tanks were recirculated frequently and an artificial polyethylene substrate was installed to increase available surface area by approximately 50%. Survival, total yield and daily growth rate of *M. rosenbergii* in the third experiment, when compared to previous studies, strongly confirmed the effectiveness of this artificial integrated system and high density culture of *M. rosenbergii* juveniles (380-400 individual/m³) during production cycle.

Table 1 Comparative survivals (%), average daily growth (ADG), yield and feed conversion ratio (FCR) of *M. rosenbergii* and plant in all polyculture systems.

Prawn/Plant	Survival (%)	ADG d ⁻¹	Yield (g/tank)	FCR
Exp. I Prawn	0-68.1	0.011-0.016	402.1-820.0	0.36-0.8
Plant	-	-	125.2-944.8	-
Exp. II Prawn	36.7-78.3	0.012-0.036	10.6-327.1	1.40->10.0
Plant	-	-	341.9-2158.5	-
Exp. III Prawn	41.0-93.8	0.04-0.070	481.1-1343.0	0.42-1.2
Plant	-	-	84.2-1783.4	-

FEASIBILITY OF EARLY FARMING OF FRESHWATER SHRIMP *Macrobrachium rosenbergii* WITH OVER-WINTERED JUVENILES

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Substantial income increase from the constantly shrinking agricultural lands, particularly in low-lying one-crop flood lands, is a major concern to reduce rural poverty in Bangladesh. Of late, many farmers profitably have been growing freshwater shrimp *Macrobrachium rosenbergii* alternating with boro (irrigated) paddy in such lands that previously used to remain totally fallow during the second half of the year. Currently, the annual export earnings from *M. rosenbergii* is about US \$72 million, nearly 20 percent of the total export from the shrimp. With constantly increasing family sizes, resultant new needs for the added population and ever increasing living cost, the limited farm lands must quickly generate more income for farmers to meet the life's minimum needs. Farm income could be substantially increased if it were possible to raise a full crop of high priced *M. rosenbergii* in the first half of the year, in addition to the usual crop in the second half. Early farming of this shrimp is a difficult proposition as post-larvae either of hatchery or of the wild origin are unavailable during the first 3 – 4 months of the year.

Economic feasibility of raising an early and full crop of the shrimp during the first half of the year has been successfully demonstrated using over-wintered previous year's late season juveniles through the cold winter months. The experiments were conducted in farmers' ponds at two places from 29 October to 19 May. The winter temperatures during December – February ranged between 16° C and 22° C; the temperature gradually rose up to 32° C in May. The extrapolated production per hectare was over 1600 Kg in better stocked ponds, as against the national average of around 300 Kg. The gross profit ranged Tk 376,200 – 339,664 (US\$ 6,400 – 5,800) /ha as against only about US\$ 370 from boro paddy suggesting high economic feasibility of farming *M. rosenbergii* using over wintered juveniles.

Effective extension of the new technology may trigger hatchery, nursery and farm operations which in turn will increase production, sales and incomes leading to poverty reduction.

Table Summary of stocking and extrapolated production and gross profit per hectare

Parameters	Pabna District	Mymensingh District	
	Pond 1	Pond 1	Pond 2
Pond area (m ²)	350	767	476
Experiment period	29.10.00 – 16.05.01	25.10.00 – 19.05.01	25.10.00 – 19.05.01
Shrimp initial av. wt. (g)	6.8	0.9	0.9
Stocking /m ²	2.86	1.43	3.57
Final av. wt (g)	74.72	61.96	60.17
Survival (%)	79.5	77.18	75.71
Production (Kg.) / Ha	1,695	685	1,626
Production cost (Tk) / Ha	268,714	89,152	188,802
Sale (Tk.) / Ha	644,914	222,881	528,466
Gross profit (Tk.) / Ha	376,200	133,729	339,664

INTEGRATED AGRICULTURE- AQUACULTURE (IAA): A WAY TO AQUACULTURE SUSTAINABILITY IN BHOLA ISLAND, BANGLADESH

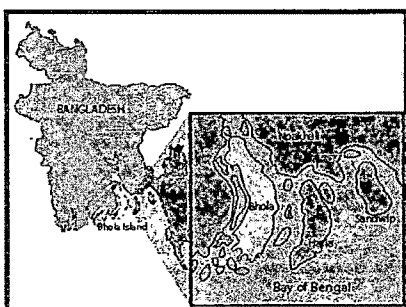
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Bhola, a coastal island of Bangladesh, is different from the rest of the country not only because of its unique geo-physical characteristics but also for its coastal conditions and vulnerabilities. About 1.5 million people live in Bhola, of which the majority is poor. Per capita income of the islanders is Tk. 18,506 compared to the national average of Tk. 22,295. Traditionally and historically, peoples' livelihoods center on natural-resource-based activities. However, due to increasingly extraction of natural resources, livelihood options are becoming limited and dwindling faster.

As an alternative livelihood, the islanders are involved in cultivating crops, livestock and fish to some extent. The biggest constraint in agriculture is the scarcity of land, which declined from 0.13 ha per capita in 1960 to 0.06 ha in 1996. In this context, fish culture offers an opportunity, as there are about 60,000 ponds covering a total water surface of 4500 ha. To support aquaculture in Bhola, the WorldFish Center, with funding support from USAID, initiated an aquaculture extension program in partnership with local NGOs. In 2002, 250 ponds were altered for different aquaculture practices e.g. carp polyculture in ponds and rice fields as well as fish nursery. To facilitate the uptake of improved aquaculture practices by resource poor households, integration of agriculture with aquaculture (IAA) is emphasized.

Farmers are given full freedom to choose aquaculture system based on their available resources. It is found that 70% of the smallholders, in general the better-off farmers adopted non-IAA systems in perennial ponds based on off-farm inputs and 30% essentially resource-poor smallholders preferred aquaculture systems integrated with on farm available resources including dike cropping (IAA).

Both IAA and non-IAA ponds were stocked at a density of 1 fish per m² with 6 species of Indian and Chinese carps. Integrated systems produced on average 1.2 t and 3.5 t. ha⁻¹, worth Tk 72,000 and 35,000 for fish and vegetables respectively. . Non-integrated ponds produced 1.5 t.ha⁻¹, fish worth Tk 90,000 but needed higher investments. . In conclusion, integrated systems generated 27% more income.



Location of Bhola Island



Dike Cropping



Dike cropping: Bottle gourd

COMPARATIVE STUDY WITH ON-FARM TRIALS OF ORGANIC & INORGANIC FERTILIZATION REGIMES ON CARP SPECIES IN TWO DIFFERENT AREAS OF BANGLADESH

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Aquaculture and fisheries play an important and vital role in the national economy of Bangladesh in terms of employment, income, nutrition and foreign exchange earning. Out of 140 million people of Bangladesh, 12 million people are engaged in aquaculture and fisheries related activities. Bangladesh has a number of aquaculture related projects that have been funded by international donor organizations. Proshika, national NGO, has been implementing aquaculture and fisheries development activities independently in Bangladesh through its extension network by group members and sometimes in collaboration with other agencies.

On-farm trial was conducted in Bangladesh in two different ecological zones from July 2002 to March 2003 in collaboration with Bangladesh Agricultural University (BAU) with the guidance of Asian Institute of Technology (AIT), Thailand. The study compared the effect of fertilization regimes suggested by Proshika (500 kg/ha quick compost as organic fertilization on weekly basis) and BAU (1250 kg cow dung, 31.25 kg urea and 13.625 kg TSP per ha on fortnightly basis) on water quality, fish production and economic return. On-farm trials were conducted in 12 ponds of average size 0.12 ha each, six with organic fertilization and 6 with organic and inorganic fertilization. The ponds were stocked with seven fish species: silver carp (*Hypophthalmichthys molitrix*), catla (*Catla catla*), rohu (*Labeo rohita*), grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), mirror carp (*C. carpio communis*) and mrigal (*Cirrhinus mrigala*) at a density of 10,000/ha in the ratio of 11:6:11:2:3:2:5.

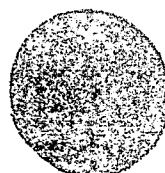
The highest gross fish yield of 5.4 t/ha were achieved through organic fertilization while lowest gross fish yield of 5.0 t/ha were achieved through organic and inorganic fertilization. While highest revenue of Tk. 148,459/ha/year was achieved from organic fertilization, organic + inorganic fertilization yielded lower revenue of Tk.146,247/ha/year. However, highest net return was in case of mixed fertilization. Considering the cost of fertilization, fish production and economic returns, on-farm trials indicated that for carp polyculture, mixed fertilization is slightly better as compared to only organic fertilization 1250 kg cow dung, 31.25 kg urea & 13.625 kg TSP per hectare with combination of organic & inorganic both fertilization regime on fortnightly basis.

POLYCULTURE OF *Pangasius hypophthalmus* WITH INDIAN MAJOR CARPS FOR HIGHER FISH PRODUCTION

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An experiment was conducted in 12 ponds of 0.02 ha each at Bangladesh Agricultural University, Mymensingh for 140 days to evaluate the effect of introduction of *Pangasius hypophthalmus* on the growth and production in polyculture with Indian major carps: *Catla catla*, *Labeo rohita* and *Cirrhinus cirrhosus*. Four polyculture-stocking patterns were tested each with three replications. The stocking combinations were: 4940 *C. catla*, 2470 *L. rohita*, and 2470 *C. cirrhosus*/ha (T_1); 2470 *P. hypophthalmus*/ha (T_2); T_1 + 4940 *P. hypophthalmus*/ha (T_3); and T_1 + 7410 *P. hypophthalmus*/ha (T_4). All experimental ponds were fertilized every 10 days with triple superphosphate, urea and cow dung at the rate of 24.70, 24.70 and 617.5 kg/ha, respectively. Pelleted feed was given only for *P. hypophthalmus* at the rate of 8-4% of the body weight per day, half in the morning and the other half in the afternoon. Addition of *P. hypophthalmus* to polyculture of major carps was found to exert an influence on qualitative and quantitative production of phytoplankton as well as on water quality. Phytoplankton biomass was higher in ponds with high *P. hypophthalmus* density where higher amount of feed was supplied. Growth of *C. catla* and *L. rohita* was found higher in ponds where *P. hypophthalmus* was stocked. This was possibly due to higher production of plankton in these ponds and partial share in feed supplied to *P. hypophthalmus*. Maximum growth of *C. catla* and *L. rohita* was observed in T_4 (with 7410/ha *P. hypophthalmus*) which was not significantly higher than the growth of T_3 (with 4940 *P. hypophthalmus*/ha). Growth of these two species was also good in T_2 . Both *C. catla* and *L. rohita* showed their lowest growth in T_1 where *P. hypophthalmus* was not stocked. Influence of *P. hypophthalmus* on the growth of *C. cirrhosus* was not significant. Growth of *P. hypophthalmus* was found to vary with the stocking density. The best growth of *P. hypophthalmus* was found in T_3 (4940/ha). Significantly higher total fish yield ($p < 0.05$) was found in treatments where *P. hypophthalmus* was included. Total carp yield was highest in T_4 , followed by T_3 , T_2 and T_1 . In the present study, though addition of *P. hypophthalmus* at densities of 2470, 4940 and 7410/ha in polyculture of major carps seemed to be feasible and profitable, a density of 4940/ha (T_3) is recommended for higher production and profit.

Promoting Environmental Friendly Aquaculture



FAN IMPROVED CULTURE ENVIRONMENT WITH A DEVELOPED CLOSE-CYCLE AQUACULTURAL SYSTEM & DESIGN FOR INTENSIVE CULTURE OF TILAPIA

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This study was conducted to develop and test the aquacultural water recycling system and design and its capability to provide a conducive culture environment for the intensive culture of *Oreochromis niloticus*, at three different stocking densities of three treatments with two replicates, respectively (46 fish/m³, 57 fish/m³ and 68 fish/m³). The design comprises of nine individual units and each unit with its own individual configuration. The units are: 1) fish tanks (42 m³ volume of water), 2) collector tank cum primary sedimentation unit, 3) secondary sedimentation unit, 4) down-flow sand filter (0.9 m³ volume of media), 5) trickling filter (0.9 m³ volume of media and media surface area of 148.79 m²), 6) denitrification unit, 7) gravity aerator (oxygenation & degassing unit), 8) additional oxygenation unit, and 9) reservoir (aged tap water, pre-aeration). They are constructed on an elevation with gravity flow to reduce using pumping hardware except at unavoidable places in the recycling system. All units are shaded to protect from sunlight and rain. Fish were fed daily using commercial fish feed (32% protein) during experimental trials on a 2.26% body weight basis, three times a day.

The water quality parameters were measured daily before water exchange (4% daily) and the BOD and COD was measured once a week to determine the removal efficiency of the whole system. The system's culture period was 64 days excluding 8 weeks for system acclimation period and 2 weeks for species acclimation period. The fish density of the system was 12 kg/m³ although the system's carrying capacity was 19.34 kg/m³. This paper presents the system's performance by statistical comparison of important water quality criteria, which are the most important characteristics for the system. The overall efficiency of the system based on water quality is described in Table 1.

Table 1: Water Quality of the Recirculatory Aquacultural System and Design during the Culture Period ^{1,2}

Parameter	Before Run the Experiment	During the Culture Period (64 days)		Whole System's Efficiency to Removal Concentration Rate	Whole System's Efficiency to Percent Removal ³
		Fish Rearing Unit	Influent of the Fish Rearing Unit		
Biological Oxygen Demand (BOD ₅) (mg-O ₂ /L)	-	5.24±1.13 ^a	0.97±0.27 ^b	4.28	81.49 %
Chemical Oxygen Demand (COD) (mg/L)	-	34.39±2.62 ^a	11.52±1.60 ^b	22.87	66.50 %
Alkalinity (mg/L as CaCO ₃ Total (T or M) Alkalinity)	-	55.20±4.63 ^a	54.80±2.07 ^a	0.40	0.73 %
Carbon Dioxide (CO ₂) (mg/L)	-	23.75±1.36 ^a	8.42±0.88 ^b	15.33	64.55 %
Nitrite (NO ₂ ⁻ -N or NO ₂) (mg/L)	-	0.72±0.03 ^a	0.20±0.00 ^b	0.52	72.22 %
Total Ammonia Nitrogen (TAN) (mg/L)	0.07±0.01	0.62±0.06 ^a	0.07±0.01 ^b	0.55	88.71 %
Total Suspended Solid (TSS) (mg/L)	-	3.40±0.31 ^a	0.32±0.12 ^b	3.08	90.59 %
Dissolved Oxygen (DO) (mg/L)	5.11	5.59±0.06 ^a	7.42±0.12 ^b	1.83*	24.66 %*
Temperature (degree centigrade)	27.53	27.54±0.37 ^a	27.61±0.31 ^a	-	-
pH	7.05	6.77±0.05 ^a	7.04±0.13 ^a	-	-
		(6.7 to 6.87) [#]	(6.7 to 7.43) [#]		

¹ Mean±s.d. of three replications. The different superscript within rows is statistically different (P<0.05).

² After run the trial, in fish rearing unit was arranged few more diffuser (air stone) to increase DO.

³ Calculation is followed from Frank and Nancy, 1999.

* Whole System's Efficiency to Adding Concentration Rate and Percent Added.

[#] The range of the pH value.

INFLUENCE OF CONFINEMENT STRESS AND SEX RATIO ON THE IMMUNE RESPONSES IN *Oreochromis mossambicus*

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The ever growing desire of fish farmers to obtain maximum yield from limited area has often led to extreme crowding in farms leading to sudden outbreak of diseases and heavy mortality. To investigate the effect of crowding on the immune system of fish, outbred *Oreochromis mossambicus* were confined at three different degrees i.e., 4gl⁻¹ (control), 8gl⁻¹, 16gl⁻¹ and were maintained in three different sex ratios i.e., i) All male ii) All female iii) Equal male and female (mixed) for a period of 28 days. The fishes were assessed for their specific immune response in terms of antibody response to *Aeromonas hydrophila* assayed by ELISA, nonspecific immune response in terms of serum lysozyme activity and intracellular reactive oxygen species (ROS) production and integrated immunity by host resistance test. The monosex ratio groups showed enhancement of antibody response on confinement. Irrespective of the sex ratio of the population, the intracellular ROS production showed suppression on confinement. In all-male sex ratio group confined, there was enhancement of serum lysozyme level. The host resistance test showed the highest percent mortality in all male group. Except in ROS, there was no significant difference between the treatment groups i. e) 8gl⁻¹ and 16gl⁻¹. But all other groups showed a percent mortality of nearly equal to that of the control. The modulation observed might due the action of pheromones and social stressors like subordination stress.

Table 1 Table showing the effect of crowding in different sex-ratio groups on the peak day (a) antibody response (b) Intracellular ROS production (c) serum lysozyme level (d) Host resistance

a)				
	Antibody production (OD)	All-male	All-female	Equal male & female
a)	Control (4gl ⁻¹)	0.853±0.3	0.977±0.2	1.314±0.4
b)	8gl ⁻¹	1.211±0.4 ^a	1.221±0.7 ^a	1.225±0.2
c)	16gl ⁻¹	1.282±0.4 ^a	1.202±0.8 ^a	1.355±0.4
b)				
	Reactive Oxygen Species production (OD)	All-male	All-female	Equal male & Female
a)	Control (4gl ⁻¹)	0.632±0.02	0.564±0.07	0.523±0.01
b)	8gl ⁻¹	0.394±0.04 ^a	0.396±0.04 ^a	0.414±0.05 ^{ac}
c)	16gl ⁻¹	0.275±0.08 ^a	0.291±0.08 ^a	0.249±0.04 ^{ab}
c)				
	Serum lysozyme Level (units/ml)	All-male	All-female	Equal male & female
a)	Control (4gl ⁻¹)	837.5±77	881±32	735±54
b)	8gl ⁻¹	1045±101 ^a	906±43	628±16 ^a
c)	16gl ⁻¹	1192±83 ^a	851±26	542±29 ^a
d)				
	Host resistance (Percent % mortality)	All-male	All-female	Equal male & female
a)	Control (4gl ⁻¹)	30	23.3	20
b)	8gl ⁻¹	40	30	33.3
c)	16gl ⁻¹	56.6	36.5	40

Significant ($P < 0.05$) when compared with ^a the control (4gl⁻¹), ^b 8gl⁻¹ and ^c 16gl⁻¹

NEW PARADIGM IN FARMING OF FRESHWATER PRAWN *Macrobrachium rosenbergii* WITH CLOSED AND RECYCLE SYSTEMS

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An experiment was conducted in 15 cement tanks (2 x 2.5 x 1) m at the Asian Institute of Technology, Thailand, during 5 January-12 May 2004, to develop closed and recycle systems for culture of giant freshwater prawn (*Macrobrachium rosenbergii*). Juvenile prawns were cultured in three systems as three treatments, each in triplicate: (A) open system with water exchange, (B) closed system with aeration, and (C) recycle system, in which water from a prawn tank was circulated through a Nile tilapia (*Oreochromis niloticus*) tank to a water mimosa (*Neptunia oleracea*) tank and back to the prawn tank. Prawn juveniles of 2.8 g were stocked in all prawn tanks at an average density of 19 prawns m⁻², sex-reversed Nile tilapia of 10.6 g were stocked in tilapia tanks at 2 fish m⁻², and water mimosa seedlings at 0.4 kg m⁻² were planted in mimosa tanks. Prawns were fed *ad libitum* two times daily.

Survival of prawns, ranging from 40.64% to 88.72%, was highest in the closed system, intermediate in the recycle system, and lowest in the open system ($P < 0.05$). There were also mass mortalities in one open and one recycle tank. Growth of prawns was not significantly different among all three systems ($P > 0.05$), while gross and net yields of prawn were significantly lower in the open system than in closed and recycle systems ($P < 0.05$). Feed conversion ratio (FCR) in the open system was 2.81, which was significantly higher than in the closed (1.67) and recycle (1.78) systems ($P < 0.05$). Prawn recovered 12.02% N and 7.01% P from feed and fertilizer in the open system, and 25.26% N and 13.67% P in the closed system. Prawn, tilapia, and water mimosa together recovered 39.55% N and 25.53% P in the recycle system. Economic analyses showed that there were no significant differences in net returns among the three systems.

The present study demonstrated that the closed and recycle systems are more environmentally friendly and have good profit potential compared to the open system. Further study is needed to determine appropriate ratio of culture area for prawn, tilapia and mimosa to minimize environmental pollution, optimize production and maximize profits in the recycle system.

**COVE CULTURE OF MARBLE GOBY *Oxyeleotris marmorata* BLEEKER AND CARPS
IN TRI AN RESERVOIR OF VIETNAM**

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This study was conducted in the 5.91-ha Truong Dang Cove of Tri An Reservoir of Vietnam during June 2002–May 2003 to describe cove culture of marble goby (*Oxyeleotris marmorata* Bleeker) with silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*) stocked at 0.096, 0.047, 0.047, 0.047 and 0.017 fish/m², respectively. After 6.5 months of culture period from October 2002 to May 2003, the gross yield of marble goby was 251.1 kg/ha/crop, with mean weight of 353 g/fish, while gross yields of silver carp, bighead carp, common carp and grass carp were 90.5, 114.3, 84.6 and 35.0 kg/ha/crop, with mean weights of 333, 368, 301 and 321 g/fish, respectively. Survival was 73.7 % for marble goby and 55.1–62.8 % for carps.

Results indicated that the cove had relatively good physico-chemical conditions with diversified natural food sources such as terrestrial vegetation, phytoplankton, zooplankton, benthos, detritus, small wild fish and prawns. The major food item of marble goby was small freshwater prawns, followed by small wild fish and benthos. Addition of marble goby to carp polyculture in cove gave much higher economic return compared to that without marble goby (US\$2,713.2 vs. US\$260/crop). This study has demonstrated that cove culture of marble goby is a prominent prospect ecologically, technologically and economically.

INTEGRATED CAGE-CUM-POND CULTURE SYSTEMS WITH HIGH-VALUED SAHAR *Tor putitora* IN CAGES AND LOW-VALUED CARPS IN OPEN PONDS

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The concept of integrated cage-cum-pond culture system is to culture high valued fish with feeding in cage and low valued fish to culture in open pond with natural feed derived from the waste released from cage. This experiment was conducted in 15 earthen ponds of 100 m² in surface area and 1.2 m in depth at the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal, for 150 days to test the feasibility of Sahar (*Tor putitora*) in cages and carps in open ponds. In this integrated cage-cum-pond system, each cage with dimensions of 1.5 m x 1.5 m x 1 m, equivalent to a water volume of 2-m³ was suspended in each of the treatment ponds. There were one control and four treatments with three replicates each namely: carps at 1 fish m⁻² in open ponds without cages (control, T1); Sahar at 5 fish m⁻³ in the cage and carps at 1 fish m⁻² in open ponds (T2); Sahar at 25 fish m⁻³ in cage and carps at 1 fish m⁻² in open pond (T3); Sahar at 50 fish m⁻³ in cage and carps at 1 fish m⁻² in open pond (T4); Sahar at 100 fish m⁻³ in cage and carps at 1 fish m⁻² in open pond (T5), giving ratios of caged to open-pond fish of 0:1, 0.1:1, 0.5:1, 1:1, and 2:1. Caged Sahar were fed with a locally made pelleted feed (28% crude protein) twice daily at the rates of 3% body weight per day, while no feed or fertilizers were added into open water of the caged ponds. The control ponds were fertilized weekly using DAP and urea at rates of 4 kg N and 2 kg P ha⁻¹ d⁻¹ throughout the experimental period.

Survival of Sahar (93-100%) was high without significant difference among all caged treatments (T2 - T5). Daily weight gains of Sahar, ranging from 0.11 to 0.25 g fish⁻¹, were significantly higher at lower stocking densities, but net and gross yield of Sahar increased significantly with increasing stocking density. Feed conversion ratio (FCR) of Sahar ranged from 2.2 to 2.8, and was not significantly different among all treatments. The total net and gross yields of all carps in open pond were not significantly different among caged treatments (T2- T5), but the control treatment (T1) resulted significantly higher carp yields than in cage-pond integrated treatments. Moreover, the total net and gross yields of carps in the control were significantly higher than the combined net and gross yields of Sahar+carps in the all treatments. The overall FCRs in the treatments were 0.15 - 0.95, and were significantly better in the lower Sahar density treatments. The control and all treatments produced positive net returns, and the highest net returns were produced by the control, followed by the treatments from high to low stocking density of Sahar.

Results clearly showed that cage wastes were insufficient to release enough nutrients to produce natural food in open ponds even in higher Sahar to carp ratio tested in this experiment yielding lower yield of carps compared to fertilized pond without cages. However, this study has demonstrated that the high-valued Sahar has great potential to be cultured in this integrated cage-cum-pond culture system, but it is necessary to fine-tune stocking ratios of Sahar to carps through adjusting stocking density of Sahar in cages and cage size or cage number, and provide high quality feed.

EFFECTS OF DIFFERENT FEEDING REGIMES ON AMMONIA EXCRETION AND FECES PRODUCTION OF FINGERLING RAINBOW TROUT *Oncorhynchus mykiss*

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Rainbow trout (*Oncorhynchus mykiss*) is one of the most important freshwater aquaculture fish and is mainly produced from flow-through systems from which effluent discharges directly to the natural water bodies. High concentrations of organic (mainly feces) and inorganic (mainly ammonia) wastes and ammonia and feces production is directly related with amount of daily feed and feeding frequencies in the effluent have a direct impact on the natural environment. The purpose of this study is to evaluate the effects of various feeding regimes on ammonia excretion and feces production in rainbow trout.

Eleven fish, mean weight 9.3 g were stocked in each of 9 aquariums and maintained on three different feeding regimes of 1%, 2.5%, and 5% of body weight per day. Each treatment was carried out in triplicates. Fish were fed every 3 hours from 8 am to 8 pm and daily ammonia excretion was measured every 2 hours. Basal ammonia excretion was measured 4 days after feeding terminated. Daily feces excretion was measured once a day at 8 am. Feces production after feeding stopped was also measured every 2 hours for 45 hours.

Fish fed 1%, 2.5%, and 5% daily ration groups grew from an average weight of 9.3 g to 12.0g, 18.7g, and 26.5g respectively, after 4 weeks. Average ammonia excretion of the fish fed 1%, 2.5%, and 5% daily ration were 20.7mg/kg/h, 28.8mg/kg/h, and 36.5mg/kg/h, respectively and the maximum ammonia excretion were recorded 14 hours after the first feeding. The amount of ammonia excretion increased as daily ration increased. Basic ammonia excretions of the treated groups ranged from 2.7 to 2.8mg/kg/h and were not different among the feeding regimes. There was a positive correlation between daily feeding level and feces production. The maximum amount of feces was collected at 14 hours after the first feeding in all treatment groups and the amount of collected feces from 1%, 2.5% and 5% daily ration groups for 2 hours were 0.023, 0.113, and 0.354 g in dry weight. Average amount of feces from all treatment groups were decreased rapidly after stop feeding and mucus was shown in the feces 3 days after stop feeding.

INTEGRATED CAGE-CUM-POND CULTURE SYSTEMS WITH HIGH-VALUED STINGING CATFISH *Heteropneustes fossilis* IN CAGES AND LOW-VALUED CARPS IN OPEN PONDS

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An experiment was carried out for a period of 237 days at Bangladesh Agricultural University to adapt the integrated cage-cum-pond systems developed by Aquaculture CRSP to local conditions in Bangladesh, to determine appropriate stocking density of selected fish species in cages, to assess growth and production of fishes in both cages and open ponds, and to assess the economic and environmental benefits of this integrated system.

Stinging catfish and carps were stocked in cages and open water of ponds to give caged to open-pond fish ratios of 50:100, 100:100, 150:100, and 200:100 (1:2, 1:1, 3:2, and 2:1) as four treatments of three replicates each. Stinging catfish fingerlings with a mean weight of 12.6-12.7 g were stocked at 50, 100, 150, and 200 fish per 0.85-m³ cage, while fingerlings of four carps, including silver carp (*Hypophthalmichthys molitrix*), catla (*Catla catla*), rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*), were stocked at 100 fish per pond with a species ratio of 2:2:3:3 in open water of all ponds. Commercial pelleted feed (30% crude protein) was given to caged fish twice daily at a rate of 10% body weight per day. No feed or fertilizer was added into open water of the ponds.

Survival of caged catfish was low, ranging from 39.33% to 60.67% with the highest survival in the 100:100 treatments. Caged catfish in all treatments grew slowly, giving daily weight gains of about 0.06 g fish⁻¹. Net yields in the 50:100 and 100:100 treatments were 0.10 and 0.18 kg m⁻³ crop⁻¹, respectively, while the other two treatments gave negative net yields. FCRs were extremely high in the 50:100 and 100:100 treatments (131 and 148, respectively), while FCRs were negative in the 150:100 and 200:100 treatments (-66 and -311, respectively). Survival of open-pond carps was high, ranging from 71.67% to 100% without significant difference for each carp species among all treatments. All carp species grew steadily throughout the experimental period, with daily weight gains of 0.76 – 1.62 g fish⁻¹. Net and gross yields of all carps were significantly higher in the 100:100, 150:100 and 200:100 treatments than those in the 50:100 treatments. Overall FCRs were best in the 200:100 treatment (0.42), intermediated in the 100:100 and 150:100 treatments (0.76 and 0.59, respectively), and poorest in the 50:100 treatment (0.86). Net revenues were positive in all treatment. This experiment has demonstrated the potential of the cage-cum-pond integrated culture system, but more research is needed.

CODE OF CONDUCT GUIDELINES IN DESIGNING TWO AQUACULTURE FACILITIES

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The World Commission on Environment and Development (1987) and the Earth Summit (1992) stressed the importance of sustainability. Various NGOs and governments have developed working papers and policies such as ecologically sustainable development (ESD). Working papers are being prepared such as the Code of Conduct for sustainable fisheries and aquaculture. Two facilities were designed, one in Australia (1997) and the other in Indonesia (2002). The design features included conservation issues, translocation issues, aquacultural wastewater treatment, by-product capability, occupational health, safety & welfare guidelines and fish quality standards as required by HACCP and WTO.

For Australia, the aim was to build an aquaculture facility under strict quarantine guidelines as required by the South Australian Department of Fisheries and AQIS. In South Australia, quarantine culture is required for barramundi (*Lates calcarifer*) and other alien species. The facility cultured barramundi and other translocated fishes. Fish entering the facility required a pathogen-free certification issued by a recognised veterinary service in Australia. Pathogen-free certification was required prior to any fish leaving the facility. The barramundi fingerlings produced in the facility were supplied to farms approved by the government to culture barramundi. The facility was enclosed. Entrance into the facility required stepping into a foot-bath and handwash as sterilisation procedures. Daily monitoring of water quality and fish health was recorded. Management of staff adhered to Australian work ethics such as Occupational Health, Safety and Welfare guidelines. A bore supplied brackish water for fish culture. Both through-flow and recirculation systems were used. The aquacultural wastewater was treated with chlorine prior to discharge into a wetland.

In Indonesia, the aim was to produce fingerlings in a land-based facility for stocking sea cages. The facility produced fingerlings for a livelihood scheme. Transmittable fish pathogens for the targeted fish species occur in the region. The fingerlings produced should be robust and have developed sufficient immunity response to adapt to the stressful environmental conditions in the sea. A few of the guidelines adopted were more stringent than that practised in Indonesia and Australia because the international Code of Conduct for aquaculture was still being developed. The larval culture area is totally enclosed but the nursery area has a roof. The water supply is pumped from a point source in deep waters beyond the fringing coral reef. The aquacultural wastewater is treated with ozone initially, then flow through a zigzag treatment system prior to discharge into deep waters, beyond the coral reef. The zigzag system increased the distance of wastewater discharge to the sea from 100 metres to 1000 metres. The required guideline for Indonesia and Australia were 60 metres and 500 metres from the nearest water source, respectively. Conservation practices were placed in the tender document for construction of the facility as contract requirements.

Both the wastewater treatment systems have the capability for by-product functions. One of the conditions for the aquaculture approval in Australia was to rehabilitate the premises from its previous commercial activity. Yabbies was introduced into the wetland. The nutrient-enriched wastewater resulted in a vibrant growth of plants and a sanctuary for aquatic birds and native animals. Foxes made the wetland its home. The zigzag system can be stocked with marine organisms as broodstock. The outflow water will carry the fertilised eggs as recruitment in the neighbouring coastal waters. The discharged water can be reused for the hatchery and culture of seaweed.

MODELING AND COMPUTERIZING VISUAL ASSESSMENT OF AN ARTIFICIAL INTEGRATED AGRICULTURE - AQUACULTURE SYSTEM

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Marketable harvest and large cost savings have been achieved in the production of aquatic animals and crustaceans using efficient culture systems and enhanced production management strategies. Decision and prediction expert programs have received considerable attention in the agricultural community. Expert programs can also play an important role in aquaculture, which is considered as an important source of dietary protein and food in developed and developing country.

In order to increase the efficiency and sustainability of integrated aquaculture-agriculture system (IAAS), expert software was adapted through basic guideline of visual basic and database optioned from previous and related researches. The structural research involved the nutrients dynamic, biological interaction (among the species), environmental impact, management and statistical methods of planning on artificial integrated culture systems. The various subjects in IAAS have capabilities for describing different aspects of integrated production. IAAS expert program consists of one communicational interface and another sub-basic form, namely: Integrated aquaculture-agriculture programs (information and statistical methods), general information of sustainable aquaculture-agriculture systems, integrated fish farming, growth rate parameters, water quality variables, nutrients and wastewater managements. Models in IAAS are organized into different levels allowing users (aquaculture experts or farmers) to simply perform different kinds of statistical analyses based on input data, available database and output resolution requirements.

INDIGENOUS TECHNICAL KNOWLEDGE (ITK) IN THE CONSERVATION OF COASTAL BIODIVERSITY IN BANGLADESH

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The coastal resources of Bangladesh are being depleted by intrusion of saline water and over-exploitation. These losses are having a negative effect on the lives of people in coastal areas. To rehabilitate and conserve these valuable resources, there is a need to gather indigenous technical knowledge (ITK) and improve the practices at Munshiganj union of Shyamnagar upazila in Satkhira district. Eleven types of ITK were identified using a questionnaire and PRA tools. Three of them, all potential farming practices (potato-sweet gourd; sea bass-tilapia; *Macrobrachium-Scatophagus*-rice-vegetables), were selected and tested scientifically at demonstration plots.

Culture of potato-sweet gourd resulted in production rates of 13,000 kg/ha of potato and 5,000 kg/ha of sweet gourd with a farm gate price of 108,000 taka/ha (US\$ 1,844/ha) and inputs costs of 46,153 taka/ha (US\$ 788/ha). For sea bass-tilapia farming, 0.16 ha produced 400 kg of sea bass and 100 kg of tilapia with a cost benefit ratio 1:2.52. The *Macrobrachium-Scatophagus*-rice-vegetables culture produced 440 kg of *Macrobrachium*, 88 kg of *Scatophagus*, 1,800 kg of rice and 300 kg of vegetables from 0.4 ha of land and earned taka 198,300 (US\$ 3,386) from input costs of 56,863 taka (US\$ 971). Our paper details the practices of applied ITKs in conserving coastal biodiversity.

**REDUCTION OF PRODUCTION COST AND MANAGEMENT OF ALGAE IN PANGAS
Pangasius hypophthalmus PONDS THROUGH INCORPORATION OF SILVER CARP
*Hypophthalmichthys molitrix***

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Monoculture of pangas, *Pangasius hypophthalmus*, and composite culture of pangas and silver carp, *Hypophthalmichthys molitrix*, were carried out in similar sized earthen ponds in the Field Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh for a period of three and a half months to find out a standard stocking ratio for maintaining good water quality and proper utilization of phytoplankton as fish food as well as to reduce the cost of fish production. Ponds were stocked with three stocking ratios: 120 pangas only (T_1), 60 pangas plus 60 silver carp (T_2), and 80 pangas plus 40 silver carp (T_3) per decimal (40 m^2). The pangas was fed with a commercial pelleted diet at the rates of 8, 6 and 4% of the body weight per day during 1st month, 2nd month and rest of the culture period, respectively. The daily ration was halved; one half was given in the morning and the other half in the afternoon. The silver carps survived on plankton and did not compete with pangas for commercial feed. In pangas monoculture ponds, the average total phytoplankton (130.75×10^3 cells/l) as well as cyanobacterial (79.27×10^3 cells/l) cell densities were much higher with higher concentration of $\text{NO}_3\text{-N}$ (1.9 mg/l) and $\text{PO}_4\text{-P}$ (1.5 mg/l) than the ponds of other treatments. Inferior water quality with lower dissolved oxygen (3.84 mg/l) was found in pangas monoculture ponds (T_1) but composite culture ponds showed better water quality with higher dissolved oxygen (4.5 mg/l in T_2 and 4.19 mg/l in T_3). In ponds of T_2 , significantly higher ($P < 0.05$) growth of pangas with an additional crop of silver carp without extra cost was obtained. The production cost of per kg fish was found to be lower in composite culture of pangas and silver carp. The present trial indicates that a combination of 60 pangas and 60 silver carp per decimal can be a suitable stocking ratio for getting good fish production with lower input cost and for maintaining good water quality and algal management. More research trials on composite culture of pangas and Indian major carps at different stocking ratios and densities are suggested to reduce input cost further and to get better economic return.

EFFECT OF LIMNOLOGICAL CHARACTERISTICS ON CULTURE-BASED FISH YIELD IN NON-PERENNIAL RESERVOIRS OF SRI LANKA

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Sri Lanka possesses about 30,000 reservoirs of which at least 13,000 are non-perennial. There is potential to develop culture-based fisheries in these numerous non-perennial reservoirs which are under-utilized partly due to lack of proper scientific investigations to identify suitable reservoirs based on their trophic status. This investigation was carried out to study limnology of non-perennial reservoirs with a view to classifying them for the development of culture-based fisheries.

Forty-five non-perennial reservoirs were randomly selected to study their limnology out of which 32 were stocked with Chinese and Indian carps, tilapia and freshwater prawns at various stocking densities. Of these, 23 reservoirs could be harvested successfully. Thirteen major limnological parameters were measured during the water retention period of each of the 45 reservoirs, between November 2001 and January 2004.

Limnological studies revealed that the non-algal turbidity is prominent in these reservoirs and Carlson's trophic state index based on chlorophyll-a is appropriate for grouping them into different trophic categories (Fig. 1). Scores of principal component analysis based on Secchi disc depth, total phosphorous, chlorophyll-a, inorganic turbidity and organic turbidity have a significant correlation with total fish yield showing that they are the key factors influencing fish yield. Shoreline:area ratio (SAR) is significantly related to fish yield (Fig. 2) in non-perennial reservoirs. SAR and stocking density (Std) are related to fish yield according to the following equation.

$$Y = -693 + 4810 \text{ SAR} + 0.484 \text{ Std} \quad (r = 0.876; p < 0.001)$$

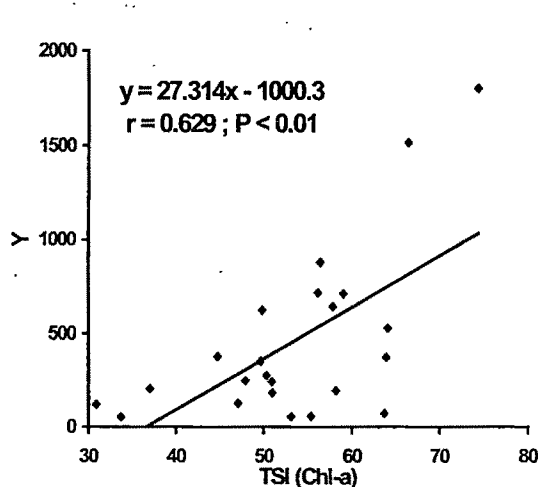


Fig. 1: Relationship between trophic state index (TSI) based on chlorophyll-a and fish yield (Y in kg ha^{-1}).

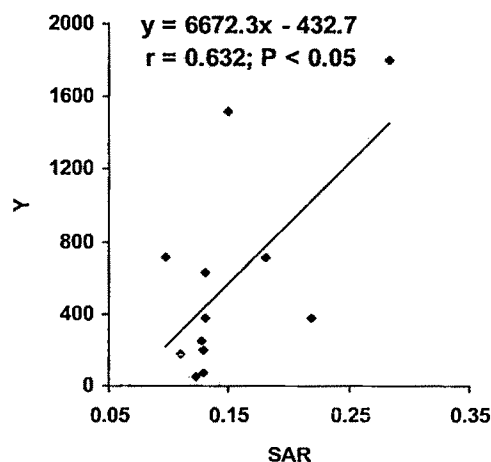


Fig. 2: Relationship of total fish yield (Y) kg ha^{-1} with shoreline:area ratio (SAR in fish yield (Y in kg ha^{-1})).

**PARTICIPATORY RESOLUTION OF WATER USE CONFLICTS IN A MIXED SHRIMP -
RICE CULTURE AREA OF THE MEKONG DELTA**

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The introduction of mixed extensive shrimp-rice culture to traditional rice growing areas of the Mekong Delta has resulted in increasing conflicts due to waste water and mud discharge after shrimp harvest and disease outbreaks. A project has been initiated to develop a participatory model for resolution of these conflicts through capacity building of existing farmer clubs in Hoa Tu 1 commune, Soc Trang Province.

A baseline survey was undertaken in May 2004 to document household economics, farmer clubs' planning, operations, and communication, and understanding about key issues of environmental monitoring and management, disease awareness and management, and shrimp culture techniques. Survey participants included all members of the focus club and cooperative being monitored through the project, club members from a non-focus club, and non-club member farmers.

All farmers interviewed operate with a high level of risk, due to high debts and low level of awareness of best culture practices. The existing clubs and cooperative have greater access to a variety of information that could assist with the improvement of rice-shrimp culture, however understanding about key issues was inconsistent within and between all four groups surveyed. The development status of the existing participatory structures, the farmer clubs, was also highly variable between the three groups surveyed.

Existing clubs and cooperatives may prove useful in developing a participatory model for wider application within the target commune, but require assistance to further develop their own cooperative capacity in the immediate future. There is need for wider education in key issues of water use, disease and shrimp culture techniques for not only club members but all commune farmers. It may be possible to address this need through a training communications network accessing all leaders within the farmer groups, village, commune, and unions. When linked with the project's task group, this network would ensure more successful two way communication between all regional water use stakeholders, from the farmers at the grassroots to the provincial authorities at the decision making level.

**REVISITING UNDERLYING ECOLOGICAL PRINCIPLES OF RICE-FISH INTEGRATED
FARMING FOR ENVIRONMENTAL, ECONOMICAL AND SOCIAL BENEFITS**

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Ecological interaction and energy and material flow in rice field in absence and presence of bottom feeding omnivore fish to give a clear scenario of food web interactions in rice field was synthesized. Rice plants, weeds, and minute algae can be competitive to each other, as they depend on common resources for growth. However, introduction of the fish about two weeks after rice plantation can add new link to the food chain by perturbation and top down control on rice competitors, pests, and mobility of nutrients towards rice plant for increased rice field productivity to benefit the farmers economically, environmentally and socially.

In relation to above synthesis, rice-fish integrated field experiments were performed in four mid hill districts of Nepal from 2000-2002. The results showed that despite of 3-5% loss of the rice cultivating area due to digging a "trench" as fish hiding place, rice yield increased up to 9%, in addition of 529 kg.ha⁻¹ fish from rice-fish integrated farming. In rice-fish system, competitive advantages of rice plants over weeds and micro algae; control of rice pests and increased nutrient supply due to top down perturbation and nutrient regeneration by fish were the reasons for increased rice yield in treatments than in controls where rice alone was cultivated.

SUSTAINABLE ORGANISATIONS OF AQUACULTURE FOR PROPER RESOURCE UTILISATION – BALANCING WATER AND SOIL CONFLICTS

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Aquaculture has been a remarkable economic growth sector in some countries and regions during the past decade. In many countries the industry has become commercially organised as a sector in its own rights.

The growth of aquaculture in India has been rather smooth when compared to that in other countries bringing forth lot of export potential as well as socio-economic benefits to the society. Developing countries like India are predominantly dependent on agriculture of their export earnings. In this marine products occupy an important position both in terms of export earnings and scope to expand this industry. At present, the impacts of aquaculture practices on environmental operations are such that which wake up violent protests from environmentalist and social activists demanding even the closure of these aqua farms. Sustainable organization of aquaculture production and reorientation it to meet social challenges in using water resources in the need of the day.

The growth rate of shrimp exports has been increased. It has increased from 2.27% in 1986-87 to 16.32% in the year of 1993-94. Comparing to the export of shrimps in terms of value shows an increasing trend except in the year 1989-90 it has 46.331 lakhs through it has increased from Rs.32, 981 lakhs in 1985-86 to Rs.1, 77,073 lakhs in the year 1993-94.

The impetus given to shrimp aquaculture is mainly due to its lucrative foreign exchange earning capacity. In India this development has coincided with unprecedented pressure on natural resources such as water and land which causes serious concern on environment. The study of coastal area affected by aquaculture and conflicts of water usage has brought out salient findings.

The water pollution problems due to discharge of untreated waste water containing large amounts of settleable solids, organic waste and nutrient load, sometimes with traces of chemicals and drugs, which would lead to hyper-nutritification and eutrophication.

Salinization of soil and water affecting livelihood requirements and agriculture and extraction of groundwater for extensive use and mixing purposes and salinization of aquifers as well as land subsidence due to reduction of aquifer capacity to hold the land.

Many drinking water sources in villages adjacent to shrimp farms have turned saline due to the indiscriminate pumping of ground water by shrimp farms. The intrusion of sea water coupled with the sewage of effluents has caused the health hazards like cholera, malaria, jaundice and eye ailments. The Bhagavathi Environment Institute, Dindigul claims that an analysis of water samples from half a dozen villages in Sirkazhi taluk has shown a disturbing degradation in the quality of groundwater. Hardness and alkalinity levels of water collected at 17 points in seven villages were alarmingly in excess of the prescribed tolerance limit for drinking water. These are attributed to shrimp farms set-up along the coast in the past two years. But CMFRI (1995) reported that the quality of groundwater in the entire coastal area (Nagapattinam District) is saline. As such implicating the very recently started shrimp farming for such development is not at all justified and that too in the absence of any data before shrimp farming came into operation for comparison.

Hence a suitable organisation of aquaculture is essential for proper water utilization. To facilitate a balanced development of aquaculture benefiting different cross sections of the population more than 50% of the water resource especially in the coastal area will be reserved for distribution to the rural poor farmers whereas the other part of resources would be judiciously allotted to the resources rich entrepreneurs or farmers for high technology-based aquaculture production. Further water pollution abatement technology must be installed with proper cleaning of ponds and technical soundness in discharging the wastes.

IMPACT OF SHRIMP CULTURE ON NATURAL RESOURCES OF MUNDEL LAKE IN SRI LANKA

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Majority of Sri Lankan shrimp farms are distributed in the North western coastal region mainly along the Dutch canal and Mundel Lake, which are the major brackish water source for the industry. The 20 year history of shrimp aquaculture has brought many changes in the resource use of Mundel Lake. The present study was aimed at investigating effect of changes on socio-economic structure in the region.

Mundel Lake was formerly used as a fishing ground and supported a good fishery of brackish water species dominated by *Nematalosa nassu* (42%) and *Arius* sp. (26%) seasonally. The shrimp catch in the 1980s was dominated by *Penaeus semisulcatus* and *P. indicus* and was seasonal while the catch data of the 1990s did not indicate any shrimp catches from this lake. Presently *P. monodon* dominates the shrimp catch throughout the year. Fish catches are dominated by *Arius* sp. (39%) and *N. nassu* (23%) seasonally but numbers have declined. The present increase in *P. monodon* catch is attributed to release of diseased shrimp from culture ponds, when disease conditions are detected at an early culture stage. The mollusc fishery in the lagoon was dominated by *Meretrix meretrix* and was seasonal. Several fisher families depended on this seasonal fishery. Presently Mollusc fishery in the region has declined by 70% and only 2 families are involved in it.

Eighteen fisher families living along the coast of the lake have been displaced as their lands have been claimed for shrimp pond construction. Forty two percent of the mangrove cover has been destroyed and 56% of the seasonal mud flats have been converted to shrimp ponds. This has caused a 40% decline in the water birds that used this habitat and 60% decline in the migratory water birds that visited the area. Agriculture in the area has declined by 28%. The major agricultural activity in the area was coconut cultivation and 22% of coconut lands have been converted in to shrimp ponds. The lake area itself has been reduced by 3% while its water capacity has been reduced by 9% according to available bathymetric data.

Other resources in the lagoon affected by shrimp culture include the sea grass cover which has depleted due to the high sediment load from effluent disposal into the system. The lagoon is connected to the Dutch canal and through it to the Puttalam and Chilaw estuaries but has no direct opening to the sea as the entrance is permanently blocked by a sand bar. Therefore recruitment of commercially important species is limited and as shrimp culture is affecting the breeding and nursery areas in the lagoon, it may seriously impact the lagoon environment and its biodiversity as well as the socio-economic status in the area in future.

INTENSIVE SHRIMP CULTURE IN ZERO AND LIMITED WATER EXCHANGE PONDS

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The production of nitrogenous waste from intensive shrimp culture is a major problem impacting the aquatic environment. As a result, environmental regulation in many countries prohibits the release of nutrient rich water into the aquatic environment. One approach to the problem is to limit water exchange in ponds and rely on heterotrophic bacteria to uptake inorganic nitrogen to produce a bacterial biomass that may contribute to shrimp nutrition. This approach needs an elemental balance of carbon-nitrogen ratio in cultivation ponds. This study investigated water quality and production variables in zero water exchange (ZWE) and limited water exchange (LWE) shrimp ponds. Results of field studies conducted at ZWE shrimp ponds in Darwin, Australia and LWE shrimp ponds in Lampung, Indonesia were compared. In both farms, organic carbon in the form molasses was used to manipulate C/N ratios. The results indicated that addition of molasses to control feed C/N ratio had a significant effect on growth and water quality, particularly for ammonia, nitrite, dissolved oxygen, total suspended solids, chlorophyll a and abundance of heterotrophic bacteria. Even though there was a higher concentration of ammonia and nitrite in the ZWE than LWE shrimp ponds, there was no significant difference in shrimp production variables. The water consumption rates estimated for both ZWE and LWE were compared with the water use in conventional shrimp farming. The level of water use in ZWE shrimp farming was found to be 8.04litre/gram compared with 8.51 litre/gram in LWE and 34-199 litres/gram in conventional shrimp farming. The new knowledge obtained in this study will assist in the future development of more efficient shrimp farming systems.

**WHO GAINS, WHO LOSES WHAT FROM SHRIMP CULTIVATION AROUND
SUNDARBANS: A CASE STUDY OF SELECTED VILLAGES OF RAMPAL UPAZILA,
BANGLADESH**

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Shrimp cultivation around Sundarbans in Bangladesh plays a vital role in the national economy due to its large contribution in foreign exchange earning. However, this contribution of shrimp farming has been undermined because of its adverse impact on income, employment, social crime and nutritional status of majority of the local people in the area. Systematic unaligned sampling was applied for selecting 44 villages and collecting information from 373 randomized respondents belonging to five landowner categories of these villages. Changes of socio-economic condition of the people based on perception of the local people and recorded documents of different studies of three time periods viz., 1975, 1985 and 1999 were consulted. This study suggests that while most of the local people in the study area were interested in shrimp than paddy cultivation in the last two decades as it were more profitable. Net income from shrimp cultivation was much higher than agriculture and recorded at Tk. 49,967 per hectare against Tk. 2704 per hectare for agriculture. The poor and marginal farmers could not cultivate shrimp in their land due to lack of capital. They were generally forced to lease out their small parcels of land to richer shrimp growers in their neighbourhood and become loser in the production process. While no men were unemployed before shrimp cultivation, 20 per cent of men and 55 per cent of women had lost their jobs after shrimp cultivation, as it is less labour intensive than traditional agro crops. Present management practices of shrimp were also found to be significantly dependent on labour from outside the Upazila. As such, the local people are again being deprived of indigenous employment opportunities. Social environment in the study area has remarkably been deteriorated mainly due to forceful occupation of land, unequal distribution of income from land and unemployment of agricultural labour forces. Average nutritional states of the majority people have decreased significantly after shrimp cultivation. Production of paddy, vegetable and fruits in the locality started falling immediately after initiation of shrimp cultivation. On the other hand productions of wheat & sugarcane have been withdrawn altogether. Moreover, production and sources of self-employment income and nutrition though cultivation of livestock had been reduced due lack of grazing land. As a result, small and marginal farmers have not only suffered economically but also lost cheap conventional source of nutrition.

**FEEDING ENHANCES ANTI- *Vibrio harveyi* ACTIVITY OF *Tilapia hornorum* IN A
SIMULATED SHRIMP CULTURE SYSTEM**

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The efficiency of *Tilapia hornorum* to control luminous bacteria in a simulated environment has been reported. However, the effect of different inputs in a shrimp culture system such as feed was not taken into consideration. This study investigated the effect of feeding on the efficiency of tilapia to inhibit the growth of luminous bacteria. Luminous bacterial count in tanks with tilapia and shrimp in both fed and unfed group were significantly lower than those in tanks with shrimp only. Counts in tanks with feeding were significantly lower than those without feeding, in both with tilapia and shrimp, and with shrimp only. Results showed that feeding enhances the antibacterial activity or improves the efficiency of tilapias to inhibit the growth of luminous bacteria *V. harveyi*.

USING ENVIRONMENTAL CAPACITY IN TROPICAL AQUACULTURE – THE TROPECA APPROACH

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Sustainable aquaculture development can bring real and lasting benefits to rural and coastal communities. But the environmental consequences of inappropriate or excessive development will adversely impact on the wider communities and the farmers themselves through poor farm performance or failure. There is therefore an increasing need for planning and management of aquaculture in developing countries. Environmental capacity is being used in some developed countries to inform the management of aquaculture as it provides a more objective basis on which to plan and regulate aquaculture operations, recognising the cumulative impacts of resource users and the assimilative capacity of the environment. The TROPECA Project, a 3-year research project (2001-2004) funded by UK Department for International Development (DFID), is investigating the potential to apply the Environmental Capacity concept to aquaculture in developing countries.

TROPECA adopted a collaborative, iterative and adaptive approach to ensure that the focus of the research is steadily refined in line with local needs. Two case studies are underway in Bangladesh (tilapia and shrimp) and three in Vietnam (shrimp x 2 and lobster). In-country researchers undertook simple mapping and sampling regimes throughout a local system for the entire culture cycles. At the same time they worked with volunteer farmers to record farmer practices and farm performance. Simple mass balance models were estimated to establish primary nutrient sources and the potential limiting factors in systems. These were then compared with farm practice and performance information before being discussed with the farmers involved. The project also sought to identify simple and locally meaningful environmental indicators to validate/test and monitor assessments and predictions made using simple environmental capacity models.

Preliminary results suggest that very simple nutrient budgets and mass balance calculations can be used to raise understanding and awareness of wider management and environmental issues, and can be used as a stimulus to more integrated and coordinated management – both within the sector and between sectors. Nutrient budgets can be undertaken at different scales, from farm pond or cage, to groups of farms, and to major aquatic systems. Consideration at these different scales helps identify critical points in the system where nutrient inputs and outputs may be seriously out of balance, and where local enrichment problems are likely to occur.

Results to date suggest that some cage culture operations have already exceeded environmental capacity, and have highlighted the need for urgent group management initiatives. In the case of more extensive shrimp systems the work has highlighted the dominance of rice agriculture in the local nutrient budget, and potential for exploiting these nutrients in aquaculture – rather than suffering adverse consequences through lack of awareness and understanding.

The final phase of the project explores the use of these results in strengthening aquaculture management frameworks, and the scale at which the assessment and associated management initiatives can most effectively be implemented.

EFFECTIVENESS OF DIFFERENT PERIPHYTON SPECIES IN IMPROVING WATER QUALITY

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In intensive aquaculture systems, nutrient concentrations, especially ammonia, nitrate, nitrite and soluble reactive phosphorous accumulates from excessive food addition and excretion of organisms reared at high density. Some of these nutrients, such as ammonia and nitrite are toxic to aquatic organisms. In consequence, organisms suffer from poor growth and reduced survival rates. Stresses the cultured organisms making them more susceptible to diseases.

A study to determine the effectiveness of different periphyton species in improving water quality was carried out in Aquatic Research laboratory, Universiti Putra Malaysia, over a 14-day period. Experiments were conducted in rectangular glass aquaria (dimension=26cm x 26cm x 41cm) containing 20 L of seawater. Locally available polyvinyl chloride (PVC) pipes were used as substrates for the experiment. Twenty pieces of PVC substrate (70 cm²) were tied to a plastic rope and hanged in to the experimental glass tank. Five treatments, each replicated three times, designating four different periphyton species (*Oscillatoria* sp., *Navicula* sp., *Cymbella* sp. and *Amphora* sp) and a control (without periphyton) were used. A known concentrations of total ammonia nitrogen (TAN) and soluble reactive phosphorous (SRP) were added into the tank water to determine the accumulation of these nutrients by different periphyton species. Variations in the physical parameters such as temperature, pH and salinity were monitored daily whereas, nutrient contents of the water (TAN and SRP) were monitored once every two days until the ammonia concentrations reached acceptable levels for aquaculture purposes.

Periphyton biomasses on the PVC substrates were analyzed twice during the whole experiment period (the first day and last day). Results indicate that there were no significant differences amongst the treatments with respect to physical parameters of the water. However, treatments with periphyton species showed significantly lower TAN concentration ($p < 0.05$) compared to the control throughout the experimental period. By day 14, the levels of ammonia in all treatments except the control were reduced to >50%. Total ammonium nitrogen reached significantly lower levels (0.90 mg/l) from an initial value of 10.00 mg/l in tanks inoculated with *Oscillatoria* sp. compared to others periphytic species within 14 days. Among the treatments it was found that *Oscillatoria* sp. significantly ($p < 0.05$) reduced TAN down to 90% and SRP down to 48% for a period of 14 days. However, *Oscillatoria* sp. yielded the lowest ($p < 0.05$) biomass compared to other periphytic species. On the other hand, the SRP levels in all the tanks with diatom species increased more than 30% within the 14 day culture period. Results of this study suggest that both TAN and SRP were reduced to *Oscillatoria* sp. whereas for diatom species only TAN was reduced.

INCREASED SHRIMP BIOMASS AFFECTS EFFICIENCY, AGAINST *Vibrio harveyi*, OF FISH CUM SHRIMP INTEGRATED CULTURE SYSTEM

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Two experiments to determine the effect of increased shrimp biomass on the growth of luminous bacteria were done. Three-ton concrete tanks covered with plywood painted black filled with micro-filtered seawater (24ppt) were used in the experiment. Tanks were stocked with *Penaeus monodon* at two different biomasses, 80 and 110g/m³, and either *Epinephelus coioides*, *Chanos chanos* and *Tilapia nilotica* (biomass=500g/tank). Tanks were inoculated with *Vibrio harveyi* to an initial concentration of 10³ cfu/ml. In tanks stocked with shrimp biomass of 110g/tank, luminous bacteria were not detected in tanks stocked with *T. nilotica* from day 22 until termination on day 28. *E. coioides* and *C. chanos* did not inhibit the growth of luminous bacteria in a shrimp culture system with shrimp biomass of 110g/m³. In tanks stocked with shrimp biomass of 80g/m³, luminous bacterial count was significantly reduced at days 5-15 in tanks with tilapia and at days 10-15 in tanks with grouper. Luminous bacteria was not observed in tanks with milkfish on day 14 but was observed on day 15. Results showed that an increase in shrimp biomass reduces the efficiency of the presence of tilapia in controlling the growth of luminous bacteria.

**PRESENCE OF SNAPPER, SEABASS, AND SIGANID INHIBITS GROWTH OF
LUMINOUS BACTERIA IN A SIMULATED SHRIMP CULTURE SYSTEM**

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This study investigated if the presence of commercially valued marine fishes such as seabass, snapper and siganid could affect the growth of luminous bacteria in shrimp culture water. Results showed that luminous bacterial count of water stocked with seabass, siganid and snapper are significantly lower than those without the fish. This showed that seabass, snapper and siganid are suitable alternative species for culture with shrimp to control or inhibit the growth of luminous bacteria.

THE EFFECTS OF LIGHT INTENSITY ON THE GROWTH OF DIFFERENT MARINE ALGAL SPECIES

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A study was conducted to examine the effects of light intensity on three different species of marine microalgae, *Chaetoceros calcitrans* (a diatom), *Nannochloropsis oculata* (a green algae) and *Oscillatoria* sp. (a cyanobacterium). The microalgae were exposed to eight different light intensities categorized into low ($<100 \mu\text{mol m}^{-2}\text{sec}^{-1}$), medium ($100\text{--}200 \mu\text{mol m}^{-2}\text{sec}^{-1}$) and high ($>200 \mu\text{mol m}^{-2}\text{sec}^{-1}$) in Environmental Test Chamber supplied with cool white fluorescent lights. The temperature of the growth chambers were maintained at $28.0 \pm 0.2^\circ\text{C}$. The Growth of the algae was measured in terms of fluorescent units using Turner Design Fluorometer (TD-700) at intervals of 12 hours. *Nannochloropsis oculata* showed the highest growth rate ($\mu_{\text{max}} = 0.09$), followed by *C. calcitrans* ($\mu_{\text{max}} = 0.08$). However, the differences were not significantly different ($P > 0.05$). The cyanobacterium attained a significantly lower ($p < 0.05$) specific growth rate ($\mu_{\text{max}} = 0.05$) compared with other algal species. This study illustrated that the growth of diatom and green algae increased with the increased light intensity, whilst the cyanobacterium did not respond well to the increased of light intensity, indicating that the cyanobacterium may need different light source with different light spectrum.

Table 1 Specific growth rate (μ) + standard error of the different microalgae; *Chaetoceros calcitrans*, *Nannochloropsis oculata* and *Oscillatoria* sp. grown in different light intensities. Mean with different superscript represent $p < 0.05$

Light Intensity	<i>C. calcitrans</i>		<i>N. oculata</i>		<i>Oscillatoria</i> sp.	
	mean	+stderr	mean	+stderr	mean	+stderr
Low ($<100 \mu\text{mol m}^{-2}\text{sec}^{-1}$)	0.01	± 0.00 a	0.01	± 0.00 a	0.03	± 0.00 a
Medium ($100\text{--}200 \mu\text{mol m}^{-2}\text{sec}^{-1}$)	0.06	± 0.01 b	0.05	± 0.00 a	0.04	± 0.00 a
High ($>200 \mu\text{mol m}^{-2}\text{sec}^{-1}$)	0.06	± 0.01 b	0.07	± 0.01 b	0.03	± 0.01 a

PLANT-DERIVED IMMUNOSTIMULANTS – AN ATTRACTIVE ALTERNATIVE TO VACCINES IN FINFISH AQUACULTURE?

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With intensification of aquaculture operations in India and other Asian countries, there has been significant increase in the incidence of piscine microbial diseases. The industry faces substantial annual loss of aquaculture production as result of microbial diseases and disease- outbreaks. Thus, there is an urgent need to tackle the situation by developing methods to prevent the disease occurrence. Vaccination of fishes is an attractive and effective method for prevention of diseases. However, since many microbial pathogens like *Aeromonas hydrophila* are known to exhibit extreme antigenic heterogeneity, development of bacterial whole organism vaccines for global or even national application seems not possible in immediate future. DNA and other genetic engineering- based vaccines, if and when developed may possibly be expensive. In the context of difficulty in developing and administering effective fish vaccines and the negative aspects of using antibiotics and other chemicals, the use of immunostimulants is viewed as an attractive alternate prophylactic measure to prevent fish diseases in culture situations.

Immunostimulants are substances which enhance the nonspecific defense mechanisms as well as specific immune responses if the treatment is followed by infection or vaccination. Fish for their disease resistance, depend more on nonspecific immunity than mammals do. Being primarily a stimulator of nonspecific immunity, immunostimulants work against wide variety of infections unlike vaccines which are highly specific. Various types of immunostimulants for fish have been investigated and reported. They include bacterial and fungal derivatives, animal extracts, synthetic chemicals, metals and vitamins. Herbs have long been used in certain regions of the world for prevention and treatment of human diseases. Traditional Indian system of medicine (Ayurveda) has identifies many plants a 'Rasayanas' which are claimed to enhance body's resistance. Recently products extracted from these medicinal plants have been reported by Indian and international laboratories to modulate immune responses in mammalian species. Also, there were reports on the nature of immunostimulatory compounds isolated from these plant sources. Both high molecular weight compounds (HMW) and low molecular weight compounds (LMW) in plants have been identified and reported to have immunostimulatory properties.

There were not many reports on plant extracts as immunostimulants in fish. This presentation aims at presenting some of the major findings of the investigations made in this laboratory on selected medicinal plants for the Immunostimulatory properties of their leaf extracts to highlight the potential of these plant- based immunostimulants in the health management of finfishes.

Plant products as immunostimulants have many advantages over other types of immunostimulants. Being plant products, the extracts are eco-friendly as they are easily biodegradable (hence even irrelevant or over use of plant extracts by farmers as they do with pesticides, will not pollute the environment). If the raw materials are available, these immunostimulants can be relatively inexpensive and so will be cost effective. Results to be discussed will indicate immunostimulatory properties of the extracts of some of these medicinal plants and the scope of using extracts of leaves and possibly other parts of these (and perhaps other) medicinal plants as immunostimulants in intensive finfish aquaculture.

IMMUNOMODULATORY AND ANTIBACTERIAL ACTIVITY OF *Eclipta alba* LEAF EXTRACTS IN *Oreochromis mossambicus*

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The effect of crude, water soluble and hexane fractions of *Eclipta alba* on (i) the specific immunity (antibody response) and non-specific immunity (serum lysozyme activity) and (ii) disease resistance against *Aeromonas hydrophila* was investigated in *Oreochromis mossambicus*. The minimum Inhibitory concentrations of the leaf extract of *E. alba* against *A. hydrophila*, and *Edwardsiella tarda* were determined to evaluate their antibacterial activity. The extracts and antigens were administered through intraperitoneal route. Bacterial agglutination assay and lysozyme assay were used to determine the specific and non-specific immune responses. Disease resistance was measured in terms of relative percent survival (RPS).

The 0.8mg crude leaf extract, 0.4mg water soluble fraction and most of the doses of hexane fraction advanced peak primary antibody titre. The enhancement of serum lysozyme activity was scattered on different days without a pattern. Fish injected with crude leaf extract at a dose of 1% were protected against experimental infection with virulent *A. hydrophila*. The water soluble fraction of *E. alba* effectively inhibited the growth of *A. hydrophila* and *E. tarda* than the crude extract. Hence the study indicates the potential of *E. alba* extracts to be used as an immunoprophylactic and a therapeutic to prevent diseases in finfish aquaculture.

Table 1 Effect of *E. alba* leaf extracts on the antibody response (Log_2 titre) in *O. mossambicus*.

Values marked by * represents significance difference at $P < 0.05$.

Dose (mg Kg^{-1})	Crude extract Mean $\pm$ S.E.	Water soluble fraction Mean $\pm$ S.E.	Hexane fraction Mean $\pm$ S.E.
0	4.7 $\pm$ 0.3	4.7 $\pm$ 0.3	4.8 $\pm$ 0.3
3.2	5.5 $\pm$ 0.2*	4.7 $\pm$ 0.2	4.5 $\pm$ 0.3
16	3.8 $\pm$ 0.2	5.8 $\pm$ 0.2*	6.8 $\pm$ 0.4*
80	4.5 $\pm$ 0.4	4.0 $\pm$ 0	6.8 $\pm$ 0.6*
400	4.3 $\pm$ 0.4	4.7 $\pm$ 0.2	7.3 $\pm$ 1.0*

Table 2 Effect of *E. alba* leaf extracts on the serum Lysozyme activity (Units/ml) in *O. mossambicus*. Values marked by * represents significance difference at $P < 0.05$.

Dose (mg Kg^{-1})	Crude extract (day 6) Mean $\pm$ S.E.	Water soluble fraction (day 8) Mean $\pm$ S.E.	Hexane fraction (day 4) Mean $\pm$ S.E.
0	716.7 $\pm$ 87.7	683.3 $\pm$ 106.3	537.5 $\pm$ 101.6
4	756.3 $\pm$ 84.4	864.6 $\pm$ 187.7	568.8 $\pm$ 72.0
40	910.4 $\pm$ 92.2	1879.2 $\pm$ 227.9*	816.7 $\pm$ 92.2*
400	1100.0 $\pm$ 117.3*	920.8 $\pm$ 211.3	864.6 $\pm$ 101.8*

EFFECT OF *Solanum trilobatum* LEAF EXTRACTS ON THE BACTERIAL PATHOGENS AND IMMUNE RESPONSE OF *Oreochromis mossambicus*

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Aquaculture is an important industry in Asia, which has the major responsibility of providing food and protein to the teeming millions of people. The disease outbreak is the limiting factor in fish culture. So aquaculturists are interested in the use of immunostimulants which has several advantages than vaccines and other chemotherapeutic agents. Experiments were carried out to study the effect of crude, water soluble and hexane fractions of *Solanum trilobatum* leaves administered intraperitoneally on the immune response of *Oreochromis mossambicus*.

The modulation of antibody response to *Aeromonas hydrophila* was evaluated in *O. mossambicus* by bacterial agglutination assay. The nonspecific immune response was assessed in terms of serum lysozyme activity. The integrated host response in terms of relative percent survival (RPS) was also assessed in the leaf extract administered fish challenged with live virulent *A. hydrophila*. The maximum RPS value of 71.42 was observed in the fish administered with 10% leaf extract of *S. trilobatum*. The minimum inhibitory concentrations of crude and water soluble fractions of *S. trilobatum* were evaluated to study the effect of antibacterial activity. The water soluble fraction was found to be inhibiting the growth of microorganisms than the crude extracts. From this preliminary study, it is clear that *S. trilobatum* could be used to promote the health status of the fish in intensive finfish aquaculture.

Table: Effect of different concentrations of *S. trilobatum* leaf extracts on the immune response of *O. mossambicus*. Significant difference (P,0.05) between treatments is indicated by an asterisk (*)

S.No	Immune response	Doses (mg Kg ⁻¹)	Fractions		
			Crude extract	Water soluble fraction	Hexane fraction
1	Antibody response (Log ₂ titre) (On day 14)	0	5.2±0.3	5.2±0.3	6.2±0.3
		6.4	6.3±0.2*	7.5±0.5*	7.3±0.5
		32	5.8±0.4*	6.83±0.4*	7.69±0.3*
		160	6.0±0*	6.83±0.3*	8.0±0.4*
		800	6.7±0.3*	-	9.0±0.3*
2	Lysozyme activity (Units/ml) (On day 4)	0	622.9±44.3	622.9±44.3	537.5±101.6
		4	1083.3±101.5*	1118.8±36.5*	922.9±96.4*
		40	1268.8±84.9*	1062.5±94.8*	854.2±92.4*
		400	1175.0±106.6*	847.9±60.7*	1222.9±129.6*

EFFICACY OF SANOSIL AS AN ANTIBACTERIAL IN FRESHWATER PRAWN FARMING

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This study investigates the efficacy of 'Sanosil', a mixture of hydrogen peroxide and silver, as an antibacterial agent in freshwater prawn *Macrobrachium rosenbergii* culture system. Sanosil is already being used as a disinfectant in drinking water, dairies, animal farms, hospital/clinics, food and beverage industry, swimming pools, pharma and chemical industry.

The minimum inhibitory concentration of Sanosil on different bacteria isolated from prawns was performed and the value was 0.003 µl/ml (Table 1). The LD₅₀ of Sanosil on PL₂₀ of *M. rosenbergii* was also conducted and was found to be 0.24 µl/ml within 24 hours. Moreover, Sanosil was very effective even at low doses and inhibited bacteria activity upto 43 hours (Table 2). Histopathology of the hepatopancreas and muscle of the *M. rosenbergii* treated with Sanosil was performed to assess the toxic effects at cellular level, if any.

Sanosil was found to be effective on different bacteria at low doses and can be used in treating water in aquafarms at the rate of 3 ppm and is good alternative to antibiotics.

Table 1: Minimum Inhibitory Concentration (MIC) of Sanosil on Different Bacteria

Bacterial species	MIC of Sanosil (µl / ml)		
	In broth	In unchlorinated water	In distilled water
<i>P. fluorescens</i>	0.04	0.01	0.003
<i>E. tarda</i>	0.04	0.01	0.003
<i>S. aureus</i>	0.04	0.01	0.003
<i>A. salmonicida</i>	0.04	0.01	0.003

Table 2: Period of Activity of Sanosil on Bacteria

Time in hours	Growth in		
	Broth	Unchlorinated water	Distilled water
Initial	-	-	-
Initial with bacteria	+	+	+
Immediately after adding Sanosil (0 hours)	+	+	+
1 to 24	-	-	-
25	+	-	-
28	+	-	-
32	+	-	-
36	+	-	-
40	+	-	-
42	+	-	-
43	+	+	+

+ Growth of bacteria

- No growth of bacteria

ETHNOBOTANICALS AGAINST VIBRIOSIS

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Luminescent vibriosis is one of the major diseases of *Penaeus monodon* and responsible for significant losses to the aquaculture industry in the Philippines and in other countries (Lavilla-Pitogo, 1990; Lightner *et al.*, 1992; Lavilla-Pitogo, 1995; Torrento, 1996; Olympia *et al.*, 2001).

Antibiotics (Baticados *et al.*, 1990) and chemotherapeutants (Baticados, and Paclibare, 1992) are traditionally used to combat these diseases but the number of antibiotic-resistant pathogenic bacteria increased and caused morphological deformities in the shrimp (Baticados and Paclibare, 1992) and adverse effects on the consumers and the environment (Jeanthon *et al.*, 1988; Le Pennec and Prieur, 1997). Continued use of chemicals causes harmful residues to accumulate not only in the flesh of fish and shrimp posing a risk to consumers' health but also in the environment. On the other hand, ethno-botanicals are plant-based drugs that are natural and biodegradable (Seraspe *et al.*, 2004).

Phytochemical screening of the plants was done in the field. The Culvenor-Fitzgerald method was used to test for the presence of alkaloids and the Froth test was used to test for the presence of saponins.

The bioactivity of the ethno-botanical extracts against *Vibrio harveyi* was conducted to identify and develop biodegradable and safe ethno-botanicals for fish and shrimp disease control. Crude methanolic extracts obtained from 16 short-listed endemic plants were subjected to vibriosis assay using the paper disc diffusion method. Three concentrations were used, namely, 100, 300 and 500mg/mL. Three replicates were run for each concentration. Chloramphenicol was used as the positive control and methanol as negative control. The anti-microbial index was calculated by subtracting the diameter of the disc from the measured diameter of the clear zone of inhibition divided by the diameter of the disc.

All of the 16 plants studied exhibited anti-vibriotic activity based on the anti-microbial indices obtained with *Vibrio harveyi* as the test organism. This is shown in the following table.

PLANT Extract	Mean of ANTI-VIBRIOTIC INDICES at concentrations of		
	100mg/mL	300mg/mL	500mg/mL
1. Biab (<i>Stephania</i> sp.)	3.11	3.67	3.44
2. Tabong (<i>Macaranga tanarius</i> Linn.)	2.00	2.33	2.33
3. Botolan (<i>Securinega virosa</i> Linn.)	1.72	2.05	2.00
4. Talisay (<i>Terminalia catappa</i>)	1.27	1.83	1.39
5. Buyo (<i>Piper betle</i> , Linn.)	0.27	1.11	1.44
6. Kasla (<i>Jatropha curcas</i> Linn.)	0.94	0.55	0.66
7. Bulubitu-on (<i>Barringtonia asiatica</i> Linn.)	0.61	0.83	1.00
8. Acacia (<i>Samanea saman</i>)	0.44	0.61	0.78
9. Kayos (<i>Dioscorea hispida</i> Dennst.)	0.44	0.43	0.33
10. Yellowbell (<i>Stenolobium stans</i>)	0.28	0.61	0.44
11. Pasaw (<i>Graptophyllum pictum</i>)	0.33	0.33	0.61
12. Alibutbut (<i>Tabernaemontana pandacaqui</i> Poir)	0.22	0.33	0.28
13. Noni (<i>Morinda</i> sp.)	0.14	0.28	0.28
14. Langka-langka (<i>Ficus septica</i>)	0.08	0.11	0.08
15. Sampaguita (<i>Jasminium sambac</i>)	0.08	0.08	0.08
16. Kamunsil (<i>Phitecolobium dulce</i>)	0.08	0.08	0.08

Table 1 Anti-vibriotic indices of the 16 plants

**THE HISTOLOGY, MORPHOGENESIS AND ULTRASTRUCTURE OF INCLUSIONS
CONTAINING RICKETTSIA- LIKE ORGANISMS FROM THE OYSTER *Crassostrea*
ariakensis GOULD**

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This report investigates RLO infection in the tissues of the oyster *Crassostrea ariakensis* and describes the histology, ultrastructure, developmental stage and morphological characteristics of RLOs and RLO inclusions and for the first time proposes the morphogenetic process of the RLO inclusions. In a recent systematic pathogen survey with moribund oysters aged 2-3 years collected from Hailing Bay in Yangxi County of Guangdong province during February to May and November to December in the year 2001, 2002 and 2003, a mass infection of an obligate intracellular prokaryotic, a pathogen referred to previously as a rickettsia-like organism (RLO) was found. Light microscope observation revealed that RLO prevalence occurred in about 87%(26/30) of the oysters. Highest infection intensities were recorded in two oysters, which had, respectively, > 80 and > 120 inclusions per gill section, > 40 and > 110 inclusions per digestive gland section. Most inclusions were observed in epithelial cells and connective tissues of the animals and most of the oysters have both tissue types infected. Inclusions were usually spherical (diameters ranging from 7 to 20 μm) in the epithelial and connective tissue cells of gills and mantle. In contrast, inclusions in digestive epithelial cells appeared oval (ranging from 31 $\times$ 23 to 55 $\times$ 36 μm) or irregular. Inclusions in gill tissue appeared slightly amaranth or purplish red and those in other tissues appeared red, typically acidophilic. The organisms were often round, dumb bell (undergoing binary fission), or occasionally rod shaped and ranged from approximately 0.32-0.6 μm in size. The organisms exhibited clearly recognizable ultrastructural characteristics of prokaryotic bacteria-like cells, including a trilaminar cell wall, electron-dense periplasmic ribosome zone and DNA nucleoidal structure. Two propagative modes, transverse binary fission and budding, were observed by TEM. The observations on RLO inclusion ultrastructure suggest that the morphogenetic process of RLO inclusions involves different developmental stages of RLOs and the changes of chemical constituents of the particles within RLO inclusion, and the chemical components of the particles may be different in different tissues. It explains possibly why RLO inclusions here appeared of different color and size in different tissues.

FINANCE BOUND REHABILITATION PROGRAM TO REDUCE THE RISK OF WHITE SPOT VIRUS INFECTIONS IN MEDIUM SCALE SHRIMP FARMS OF SRI LANKA

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Frequent outbreaks of white spot disease (WSD) have been identified as the main threat to the shrimp farming industry in Sri Lanka since 1996. Rapid uncontrolled expansion, self pollution, an increased pathogen population in the environment, sand bar formation at sea outfalls and siltation of water bodies have reduced the capacity to sustain shrimp farming in Sri Lanka. The total accumulated loans and interest of this sector is over SL Rs 8 billion and financing institutions have lost confidence, resulting in withdrawal of facilities given to the sector. A finance bound rehabilitation program incorporating best management practice (BMP) was introduced to farmers with the assistance of the Seylan Bank, a leading commercial Bank in Sri Lanka. The financial package amounted to SL Rs 669,000 per ½ ha pond to cover operational costs. Funds were released in several installments to ensure the implementation of BMP. Identified BMP included, improved pond preparation procedure, introduction of biosecurity, maintenance of a reservoir, better feed management practices, water conditioning, improved aeration, regular water quality monitoring and regular health checks. Sixty seven percent of the assisted ponds successfully completed the culture cycle with an average production of 1582 kg/ 0.5 ha. The survival rate was 79% while FCR recorded was 1.37. Good follow up and timely implementation of recommended BMP and availability of adequate infrastructure facilities at farm sites were found to be the main contributory factors for success. Nineteen percent of the ponds were infected within 45 days of stocking, while 13% of the ponds were harvested prematurely due to WSD outbreaks. Major disease out-breaks in the immediate vicinity of the ponds, delays in implementing the BMP and difficulties in changing farmer attitudes towards implementing certain BMP were found to be the reasons for disease outbreaks.

IRIDOVIRUS ISOLATED FROM DISEASED SEA BASS *Lates calcarifer* AND RED DRUM *Sciaenops ocellatus* CAUSING MASS MORTALITY IN MALAYSIA

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Mass mortalities of the cultured sea bass (*Lates calcarifer*) and red drum (*Sciaenops ocellatus*), occurred in Malaysian sea cages during July 2000. Both diseased fish species showed darker bodies, pale gills, and enlarged spleens. In addition, ulcers on the skin and fins were often observed without other clinical signs. From imprinted samples and histopathological observations of the spleen, basophilic enlarged cells were found in gills and spleen. These cells were characteristics of red sea bream iridovirus (RSIV) infection. Virus isolation from diseased fish using EPC, BB, FHM, SSN-1, BF-2 and a GF cell showed CPE of round and enlarged cells in the BF-2 and GF cells inoculated with kidney and spleen mixture. Furthermore, these degenerated cells were stained with FITC labeled monoclonal antibody against RSIV using indirect fluorescent antibody method. PCR products were amplified using primer sets in 959 bp *Pst* I fragment area except for the cases in which 2-F primer was used. The estimated PCR products were also observed by using primer set of ATPase gene ORF area, but amplified products was not seen by primer set of DNA polymerase gene ORF area.

The Amplified PCR product as 959 bp of *Pst* I fragment area was analyzed to compare the genome sequence of isolated iridovirus with RSIV and infectious spleen and kidney necrosis virus (ISKNV). Identical DNA sequence was shown among sea bass isolate, red drum isolate, and ISKNV. However, primer sequences of both isolations from sea bass and red drum in Malaysia and ISKNV have two and one nucleotide mismatch in 2-F and 1-R primer respectively as compared with RSIV alignment. These results suggested that the causative virus is not RSIV but similar to ISKNV, although the clinical signs of iridoviral disease causing mass mortality in Malaysia was similar to those of red sea bream iridovirus disease. It will be possible to detect these isolated viruses by using monoclonal antibody and PCR primer against RSIV, but caution should be taken in selecting the primer sets for PCR test.

ENVIRONMENT FRIENDLY MANAGEMENT OF ARGULOSIS IN BROOD CARP PONDS

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To prevent Argulosis in brood carp ponds pesticides like Sumithion, Cymbush, Marshal and Imithion etc. are being used indiscriminately by farmers in Bangladesh. Due to prolonged use of these pesticides, pond environment is deteriorating, zooplankton productivity is declining and *Argulus* sp. is developing resistance to those chemicals. In this background, our study in the brood carp ponds at Rajendrapur near Dhaka and Arabpur at Jessore reveals that use of bamboo splits in the pond indicate the presence or absence of *Argulus* sp. Regular drying or fire treatment of bamboo splits used in the ponds for collecting egg clusters of *Argulus* sp. considerably reduced the incidence of *Argulus* sp. Bamboo splits also increased periphyton production in the pond as feed for the fish. Some scale feeder like freshwater Chanda (Elongate glass-perchlet), *Chanda nama* also removes *Argulus* sp. from the carp pond though it also creates some problems by eating scales of silver carp (*Hypophthalmichthys molitrix*). Bamboo splits treatment and monitoring with periodical liming of pond has been recommended for environment friendly Argulosis prevention in brood carp pond.

PRE-DISPOSING FACTORS ASSOCIATED WITH OUTBREAK OF STREPTOCOCCAL INFECTION IN FLOATING CAGE-CULTURE RED TILAPIA IN RESERVOIRS

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Incidences of high mortalities in caged-culture red tilapia were reported annually from 2000 until 2002 especially in Tasik Kenyir, Terengganu. High mortality of more than 60% was reported from late March until June. Clinical signs observed were mainly spinning or erratic swimming behaviour together with eye abnormalities that include corneal opacity or cloudiness, exophthalmus and sometimes unilateral or bilateral sunken eyes. A 15 month study from September 2002 until December 2003, where a total number of 1095 tilapia were sampled from Tasik Kenyir and Tasik Pergau revealed high incidence (55%) of Gram positive bacteria. Twenty five percent were *Streptococcus* spp. isolated from moribund or fish showing clinical signs. *Streptococcus agalactiae* was the most common species isolated. Periodic outbreaks of Streptococcal infection with sudden high mortality of tilapia, particularly during the dry months were believed to be associated with periodical changes and fluctuations in water quality parameters namely temperature (30.5 to 32°C), dissolved oxygen (3.2 to 10.2ppm), pH (6.3 to 8.7), nitrite (0 to 0.02ppm), iron (0.0 to 1.13ppm) and ammonia levels (0.0 to 0.55ppm) as recorded in Tasik Kenyir. The water temperature was above 30°C throughout the affected months in Tasik Kenyir. *Trichodina* spp. and *Cichlidogyrus* sp. were the most common protozoans and monogeneans identified from the smears examined. Pathological examinations revealed meningo-encephalitis and cerebral congestion indicating infection of the brain which explains the erratic swimming behavior observed in tilapia. Other pathological change noted was hepatic necrosis in the liver. In addition to the changes in water quality parameters, husbandry management practiced by the cage-culture operators could also be associated with outbreak of Streptococcal infection. High stocking density of 80-100fish/m² and delayed removal of dead tilapia are believed to have aggravated fish mortalities. Losses due to mortalities at one of the three farms that suffered outbreak of streptococcal infection were estimated at US\$0.5 million.

MONOCLONAL ANTIBODIES SPECIFIC TO *Vibrio harveyi* 639

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Monoclonal antibodies (MAbs) against *Vibrio harveyi* were produced from mice immunized with killed and SDS treated highly virulent strain of *V. harveyi* 639. Fifteen MAbs were selected and can be divided into six groups according to their specificity to various proteins ranging from 8-49 kDa (Table). Some antibodies can be used for detection of *V. harveyi* with high sensitivity up to 10^4 CFU ml⁻¹ by dot blotting. Most of the selected MAbs did not show cross reactivity to other *Vibrio* spp. and various Gram negative bacteria tested. Only one MAb, VH39-4E shows slightly cross reactivity to the antigen of *Aeromonas hydrophila*. Another MAb, VH24-8H binds lightly to *V. harveyi* 1526 and can be used to differentiate *V. harveyi* 1526 from *V. harveyi* 639. Two groups of MAbs can be used to localize *V. harveyi* infection in the tissue of black tiger shrimp *Penaeus monodon* by means of immunohistochemistry. This study demonstrated the versatility of high specific immunoological tool for diagnosis of *V. harveyi* in aquaculture and can be further developed to a convenient test kit.

Specificities of monoclonal antibodies tested by dot blot, Western blot and immunohistochemistry. The underlined clones are the representative MAbs used in various tests.

Group	MAbs(type)	Dot blot (CFUml ⁻¹)	Western blot (kDa)	Immunohisto- chemistry	Remarks
1	<u>VH13-4D</u> (IgG ₁), VH14-10G, VH25- 8B (IgM)	~5x10 ⁵	49	-	
2	VH27-3G (IgG ₁), <u>VH3-3H</u> , VH28-3B, VH38-2D (IgG _{2a})	~5x10 ⁵	37,32	+	Bound to unprocessed antigens
3	<u>VH16-2A</u> (IgG _{2a})	~1x10 ⁸	37,32	+	Bound to processed antigens
4	<u>VH39-4E</u> (IgG _{2b})	~1x10 ⁶	22	-	Slightly cross reactivity to <i>Aeromonas hydrophila</i>
5	<u>VH24-8H</u> (IgG ₁)	~5x10 ⁵	8	-	Bound lightly to <i>Vibrio harveyi</i> 1526
6	VH2-3B, VH2-6A, VH21-10G (IgG ₁), VH28-8D, <u>VH29- 6D</u> (IgG ₃)	~1x10 ⁴	~8	-	Different band from VH24-8H antibody

+: strong immunoreactivity; -: no immunoreactivity

ECTOPARASITES AND BACTERIAL INFECTION IN CULTURED GOLDEN SNAPPER *Lutjanus johni* IN NORTHWEST MALAYSIA

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Hatchery-produced and imported golden snapper (*Lutjanus Johni*) cultured in an open deep sea-cage system at Langkawi, Malaysia and wild-caught golden snapper juveniles cultured in a floating cage system in Penang, Malaysia were examined during a disease outbreak in 2003. At the Langkawi farm, the observed clinical symptoms on the 5- to 6-month post-stocking snapper were whirling and erratic swimming behaviour, loss of equilibrium, exophthalmia and opacity of the eyes, multi-focal haemorrhages on the body surface and lesions like baldness (devoid of scales) on the head region above the eyes. In the present investigation, two different cages were affected, giving a cumulative mortality over a 2-month-period of 19% and 57%, respectively. *Streptococcus iniae* was isolated from 12 out of 14 fish examined. Identification of the bacteria was confirmed based on biochemical and biophysical reaction profiles. Histological examination showed severe systemic bacteremia in the brain and eyes. Granuloma formation was observed in the heart, indicating chronic infection. A systemic inflammatory cell mobilization of macrophages was also observed in spleen, liver, kidney and fat tissue, indicating the active status of this infection.

Clinical symptoms on the 4-week post-stocking snapper at the Penang fish farm were similar to that observed at Langkawi except that the affected snapper did not exhibit the atypical swimming behaviour. From the two fish sampled, *S. iniae* was not found. Histological examination showed that one fish had a severe bacterial infection with thin rod-shaped or filamentous bacteria in the lateral muscle. More samples are needed to determine if bacteria are the etiological agent involved in the disease outbreak. In this outbreak at Penang, 20-40% mortality was observed in two cages.

All fish examined at both culture sites were also infected with a large number of *Benedenia lutjani* (under the scales) and *Haliotrema johni* (on gills). At Langkawi, the infestation of *B. lutjani* in the diseased snapper was 60 times greater than that of clinically-healthy (without external symptoms) snapper, while the intensity of *H. johni* was similar between the two groups. The diseased snapper at Penang had few *B. lutjani* but had a high infestation of *H. johni*; this decreased over time. The reduced number of *B. lutjani* found at the second sampling time (1 week after the first sampling) at Penang was likely due to the repeated freshwater treatment given to the fish between both examinations.

This is the first reported case of *S. iniae* infection in golden snapper in Malaysia. The abnormal swimming behaviour observed in the fish is likely to be caused by the bacterial infection. On the other hand, the observed 'baldness' symptom is believed to be associated with a very high infestation with *H. johni*, as was reported earlier by Leong and Wong (1987; Asian Fisheries Science 1:83-90). The affected snapper probably rub against the sides of the net due to the irritation caused by the high number of monogeneans on the gills.

In some other batches of snapper with similar symptoms to those in the present Langkawi investigation, possibly also affected by *S. iniae* and monogenean infections, up to 80% mortality was observed, resulting in heavy economic losses. Better control measures are needed to prevent these bacterial and parasitic diseases in mariculture.

PATHOGENICITY OF ALPHA-HAEMOLYTIC GROUP B *Streptococcus agalactiae* IN *Oreochromis niloticus*

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Pathogenicity of *Streptococcus agalactiae* in tilapia (*Oreochromis niloticus*) was studied in both culture environment and experimentally infected condition. *Streptococcus agalactiae* isolated from cultured tilapia in Southern Thailand was Gram-positive cocci, catalase negative, alpha-haemolytic and serogroup B. Streptococcal-infected fish from both culture environment and experimental infection showed swimming abnormalities such as, swimming on its side, erratic surface or bottom swimming including serpentine movement. The other main signs were exophthalmia and cataract. Internally, splenomegaly, ascites as well as pale liver were observed. Fish experimentally infected by peritoneal injection using 1.30×10^4 CFU caused 70% mortality within 16 days and the LD₅₀ was 2.60×10^2 CFU. Haematocrit, haemoglobin, plasma protein and blood cells values of infected and moribund fish were significantly decreased (Table 1). Histopathological findings of tilapia infected with *S. agalactiae* produced a chronic disease with systemic involvement characterized by multiple necrotic foci in various tissues. The necrosis and vacuolization in the liver, granuloma in brain tissue, shrinkage of kidney glomerulus and hyperplasia of gill lamellae were observed. The exophthalmic eyes showed degenerated lens with large number of white blood cell infiltrated into vitreous humor. The similar histopathological changes were also observed in the fish from culture environment.

Table 1. Blood parameters of healthy and experimentally *S. agalactiae* infected tilapia.

Condition of fish	Haematocrit (%)	Haemoglobin (g/dl)	Plasma protein (mg/ml)	Red blood cell ($\times 10^5$ cell/mm ³)	White blood cell ($\times 10^4$ cell/mm ³)
Normal	21.91 $\pm$ 4.02 ^a (15.35-33.53)	5.80 $\pm$ 1.40 ^a (3.90-10.36)	31.30 $\pm$ 4.73 ^a (23.78-42.23)	17.80 $\pm$ 2.70 ^a (13.20-22.95)	7.18 $\pm$ 2.00 ^a (3.70-11.57)
Infected	10.90 $\pm$ 5.17 ^b (3.74-20.29)	3.22 $\pm$ 1.37 ^b (1.13-5.72)	14.61 $\pm$ 6.8 ^b (5.78-26.02)	8.51 $\pm$ 3.80 ^b (3.20-14.65)	1.85 $\pm$ 1.02 ^b (0.55-3.77)

Means within columns not sharing the same superscript are significantly different ($p < 0.01$)
n = 20, ranges is figure in parenthesis

OUTBREAKS OF KHV INFECTION IN COMMON CARP IN SUMATRA, INDONESIA

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In January 2003, initial mass mortality of cultured common carp due to KHV (koi herpes virus) with more than 90% mortality was observed at a private farm in South Sumatra province. The seed in the farm were presumptively transferred from West Java where KHV is known to occur. Affected fish showed gill rot, excess mucus production on the gills and skin, erosion/ulcer and/or sunken eyes. Following the initial mortality, KHV affected neighbouring farms causing mass mortality of common carp (*Cyprinus carpio*), but not tilapia which cohabitated with affected common carp. From February to September 2003, KHV spread to six localities in two provinces except in Lubuk Linggau. All common carp were introduced from Lubuk Linggau for culture (Table). Fortunately, several areas in Sumatra, including West Sumatra province, which is the top producer of common carp, are still free from KHV infection.

Experimental studies showed that koi herpes virus causes 100% mortality in healthy common carp cohabitated with affected fish; however, tilapia (*Oreochromis niloticus*) and striped catfish (*Pangasius hypophthalmus*) were not affected by KHV in both natural and experimental conditions.

Table Outbreaks of KHV infection in Sumatra, Indonesia

Provinces affected by KHV	Status of mortality in 2003		Presumptive transmission route	Counter measures
	First reported	Ceased or continuous		
South-Sumatra Prov.				
Lubuk Linggau	January	continuous	Transfer of seed from West Java	Change of culture species to tilapia
Bengkulu Prov. Curup	February	September	Transfer of seed from Lubuk Linggau	Seed production from survivors/ OTC administration
Argamakmur	July	August	?	-
Padangjaya	August	September	?	-
Jambi Prov. Kerinci Lake	June	July	Transfer of seed from Lubuk Linggau	Stop fish transfer from Lubuk Linggau
Lamo Lake	June	August	Ditto	Change of culture species to catfish
Sigombak lake	February July	April September	Ditto Ditto	Change of culture species to tilapia

**EFFECT OF AN INTESTINAL BACTERIUM BACILLUS CIRCULENS (PB7)
SUPPLEMENTED IN THE FORMULATED DIETS ON GROWTH, NUTRITIONAL QUALITY
AND IMMUNITY OF CATLA CATLA (HAM.)**

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Bacillus circulens (PB7), isolated from the intestine of *Catla catla*, was evaluated for use as a probiotic supplement in the diets for the fingerlings of *C. catla*. The effect of this probiotic supplement on the growth performance, feed utilization efficiency and immune response was evaluated. Catla fingerlings (avg. wt. 6.48 ± 0.43 g) were fed diet supplemented with 2×10^4 (diet C1), 2×10^5 (diet C2) or 2×10^6 (diet C3) *B. circulens* cells per 100g feed for 60 days @ 5 % of the body weight per day in two equal installments in triplicate treatments. The control diet (diet CC) was not supplemented with the *B. circulens*. All the diets are isocaloric and isonitrogenous. Diet C2 fed fishes showed significantly better growth, highest RNA / DNA ratio, a lower feed conversion ratio (FCR) and a higher protein efficiency ratio (PER) than fish fed the other experimental diets. Highest carcass protein and lipid was also observed in the diet C2 fed fishes. Intestinal protease activity increased significantly ($P < 0.05$) with increasing concentration of the bacteria where as α - amylase activity did not differ significantly beyond the lowest inclusion level.

At the end of the feeding trial, non-specific immunity levels and disease resistance of fish were also studied. Phagocytic ratio, phagocytic index and leucocrit value were highest in diet C2 fed fishes. After the feeding trial, the fishes were challenged for 10 days by bath exposure to *Aeromonas hydrophila* (AH1) [10^5 CFU ml⁻¹, 1 h and after 7 days 10^7 CFU ml⁻¹, 1 h]. Highest survival percentage was observed in the diet C2 fed fishes where as only 6.66% in control, which indicated the effectiveness of *B. circulens* at reducing disease caused by *A. hydrophila*.

Table 1 FCR, SGR, PER values of *Catla catla* fingerlings fed experimental diets. Means in the same column follow by a different superscript letter are significantly different ($P < 0.05$)

Diets	FCR	SGR	PER
CC	2.78 ± 0.18^a	1.75 ± 0.14^b	1.06 ± 0.12^b
C1	2.81 ± 0.27^a	1.70 ± 0.16^b	1.01 ± 0.07^b
C2	2.66 ± 0.21^a	1.98 ± 0.11^a	1.22 ± 0.10^a
C3	3.30 ± 0.42^b	1.55 ± 0.14^c	0.88 ± 0.13^b

IMPORTANT FISH DISEASES IN TROPICAL FRESHWATER AQUACULTURE

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During 2002 to 2003, field surveys on fish disease occurrences in freshwater aquaculture farms in Sumatra and identification of causative agents from affected fish were conducted. As a result, total 22 pathogens including a virus, 5 genera of bacteria, 13 genera of parasites and 3 genera of fungi were detected from four aquaculture fishes, common carp *Cyprinus carpio*, tilapia *Oreochromis niloticus*, striped catfish *Pangasius hypophthalmus* and sandgoby *Oxyeleotris marmorata*. Among the pathogens, koi herpes virus, *Streptococcus* sp. and *Edwardsiella ictaluri* caused severe mass mortalities in common carp, tilapia and striped catfish, respectively (Table).

Table Pathogens detected from tropical aquaculture fish, their hosts, pathogenicity and detection frequency.

Pathogens	Fish species	Target size of fish	Pathogeni- city*	Frequen- cy**
Virus				
Koi herpes virus	common carp	fingerling to brooder	+++	+++
Bacteria				
<i>Streptococcus</i>	tilapia	harvest size	++	+++
<i>Mycobacterium</i>	tilapia	fingerling	++	++
<i>Edwardsiella</i>	striped catfish	fingerling to immature	+++	+++
<i>Flabobacterium</i>	common carp, tilapia	fingerling	++	+++
<i>Aeromonas</i>	common carp	fingerling to brooder	++	++
Parasites				
<i>Tricodina</i>	striped catfish	fingerling	+	+++
<i>Amyloodinium</i>	striped catfish	larvae to juvenile	+	++
<i>Ichthyophthirius</i>	striped catfish	juvenile to fingerling	++	++
<i>Myxobolus</i>	common carp	fingerling	+	++
<i>Henneguya</i>	sandgoby	fingerling	?	+
<i>Epistylis</i>	striped catfish	larvae to juvenile	+	+
<i>Dactylogyrus</i>	common carp	juvenile to fingerling	++	+++
<i>Gyrodactylus</i>	tilapia	fingerling	+	++
<i>Lernaea</i>	striped catfish	immature to brooder	++	++
<i>Argulus</i>	common carp	fingerling to brooder	+	+++
<i>Centrocestus</i>	common carp	fingerling	?	++
<i>Bothriocephalus</i>	common carp	fingerling	+	++
<i>Glochidium</i>	common carp	larvae	++	++
Fungi				
<i>Branchiomyces</i>	tilapia	juvenile	+++	+
<i>Achlya</i>	sandgoby	fingerling to brooder	++	++
<i>Aphanomyces</i>	striped catfish	fingerling	+	+

* +++ causing mass mortality, ++ causing moderate mortality, + not causing mortality

** +++ frequently observed, ++ often observed, + sometimes observed

DIGENETIC BLOOD TREMATODES IN MALAYSIAN FISHES

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This paper is a review of work that has been carried out on the digenean parasites found inhabiting the bulbous arteriosus, and blood vessels of some Malaysian fishes. The ecological aspects of infection, morphology and life cycles of the parasites are highlighted and discussed in relation to studies on sanguinicolid infections in other parts of the world. Possible methods of rapid diagnosis, control and treatment are briefly described.

FIRST REPORT ON THE OCCURRENCE OF VIRAL NERVOUS NECROSIS IN *Lutjanus erythropterus* IN MALAYSIA

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The frequent disease outbreaks affecting marine cage culture systems have prompted fish farmers to try new species hoping to evade the problem. However, the introduced fish soon become susceptible to various existing pathogens. This paper reports the first findings of viral nervous necrosis infecting red snapper *Lutjanus erythropterus*, a popular new species introduced into Malaysia from Taiwan.

Some moribund red snappers were investigated for parasitic, bacterial and viral infection following reported mortality. The fish examined generally had a darkened body with exophthalmos. No other lesion or clinical signs were observed. Body and gill smears indicated the presence of *Trichodina* sp. Bacterial culture on TCBS showed yellow colonies of *Vibrio* spp. infecting the visceral organs such as the liver, spleen, kidney, eyes and brain. Viral nervous necrosis was detected with RT-PCR technique both in the single-step at 430 bp and the nested-step at 280 bp. Histopathological study of the brain and eyes confirmed the case as encephalopathy and retinopathy.

As the red snapper are currently imported from Taiwan, VNN screening of some imported fry prior to stocking is done. Preliminary examination of 10 fish showed negative results for VNN. There is strong possibility that the VNN infection in red snapper could have been acquired through horizontal transmission from local seabass (*Lates calcarifer*) and groupers (*Epinephelus* spp.) which tested positive for the virus in the same cage culture system. The RT-PCR technique can serve as a useful tool in the health management of cultured fish.

ELECTROLYSIS: A STRATEGY TO CONTROL NON- BENEFICIAL ALGAL SPECIES IN SHRIMP GROW OUT SYSTEMS

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Phytoplankton over blooming is a common phenomenon in intensive shrimp aquaculture. Algal die-off not only deteriorates water quality but also causes the proliferation of pathogenic bacteria, which remain as one of the serious problems in intensive shrimp cultivation. Domination of certain algal species occur unexpectedly in shrimp grow out ponds. It is believed that beneficial algal bloom is a key factor for successful shrimp production. However, there is no an effective method to control non-beneficial algal species selectively in order to increase the beneficial algal bloom in shrimp grow out ponds. Electrolysis is one of the most powerful oxidizers known; it has been applied for the disinfection of drinking water, for waste water management and for removal of ammonia from brine waters. Electrolysis technology also can be applied to control marine algal species. This paper attempts to describe the effect of electrolysis on different marine algal species, giving emphasis on algal survival and mortality.

Batch culture and continuous electrolysis experiments were conducted in the laboratory. Four different marine algal species were used for this experiment. Titanium anode (sintered with Pt) and stainless steel cathode were used as the electrodes. Constant DC power supply unit was used to supply 0.1 A current and 2.5 V voltage. Dead and live algal cells were identified by FDA and PI staining.

The results obtained in this work revealed that sensitivity of marine algae is different. The *Chlorella sp* is the most sensitive and the *Isochrysis sp* is the most resistant species among four species (Fig. 1). It is believed that *Chlorella sp* is non beneficial and *Isochrysis sp* as beneficial species in shrimp farming. Based on these results electrolysis technology can be developed to control algal over blooming to maintain beneficial algal bloom in shrimp ponds. FDA and PI staining can be successfully used to differentiate dead and live algal cells.

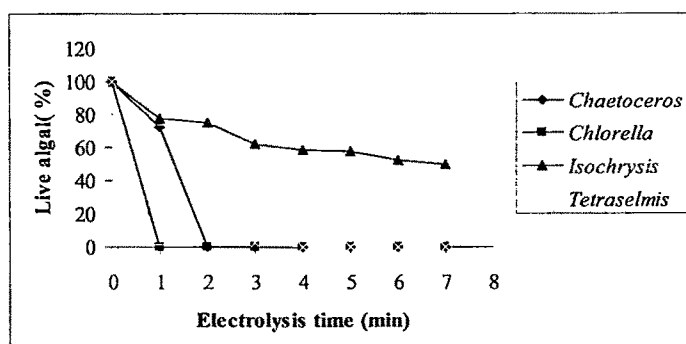


Fig. 1 Effect of electrolysis on different marine algal species

**PROTECTION OF SEABASS *Lates calcarifer* AGAINST CRYPTOCARYONIASIS BY
IMMUNIZATION WITH VARIOUS DOSES OF INFECTIOUS STAGES OF *Cryptocaryon
irritans***

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Fish bodies or gills exposed to *Cryptocaryon irritans* will result in Cryptocaryoniasis where infection of this ciliated protozoan parasite is a common problem in marine aquaculture and ornamental fish rearing in Malaysia. Despite the widespread infection occurring in hatchery, aquarium tanks and ponds, the numbers of theront *C. irritans* that lead to mortality was unknown. On the other hand, small numbers of theronts might cause the fish to survive and become a carrier which could cause disease under stressed conditions. Fingerlings of seabass, *Lates calcarifer* were immunized with varying doses of antigen of *C. irritans* and later challenged with infectious stage of *C. irritans*. Results showed that 200-400 theronts would cause 30% fish mortality within 10 days whereas 5000-10,000 theronts resulted in 100% mortality. Total mortality was also observed within 5 days in fish exposed to the highest numbers of theronts (20,000-40,000). An increase of 30% to 95% mortality within 10 days was also observed in fish exposed to 1000-2000 theronts. The results from these experiments showed that the severity dose is 20,000-40,000 theronts which caused 100% mortality within 5 days.

THE USE OF CLEANER WRASSE FOR CONTROL OF ECTOPARASITES IN MARICULTURE

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The cleaner wrasse *Labroides dimidiatus* has been shown to form a cleaning symbiosis in the marine environment with many species of marine fishes by eating their ectoparasites. This study reports on the cleaning frequency of *Labroides dimidiatus* on pompano (*Trichinotus blochi*), Red Drum (*Sciaenops ocellatus*), Sea bass (*Lateolabrax calcarifer*), Grouper (*Epinephelus* sp) and mangrove red snapper *Lutjanus argentimaculatus*. Results on reduction of sea lice infection in the cleaned fish are also presented. The potential use of cleaner fish for control of ectoparasite infections in marine cultured fish is elaborated.

STUDY ON FISH DISEASE IN HATCHERY POND AND FISH CULTURE WITH LIVESTOCK IN OUDOMXAY PROVINCE

Somphouthone Phimmachak, Saleumphone Chanthavong, Somboun

As the result from the survey in the previous year, fish disease mainly occurred in the hatchery and fishpond under integrated fish farming. therefore focusing in the hatchery and pond under integrated fish farming continued the study in 2004.

The objectives of the studies were to (1) Identify fish disease species common in hatchery and integrated farming with livestock. (2) Search the suitable techniques to prevention fish disease.

The survey was held in 4 Districts:(Hun, Pakbeng, Xay and Namor), total 14 families in hatchery 6 families and fish culture with-livestock 8 families, fish disease(parasite) were found out in 12 families (86%).

Occurring fish diseases cause and spread disease from seed, source of water and non ponds preparation before culture. Mostly occurrence of disease with fish fry and fingerling in nursing cause from parasite particularly *Lernaeasis* disease were made high mortality.

Specimen preservation was collected for diagnostic examination in local laboratory by fish be alive look for parasite and sampling techniques are used fix fish parasites by 10% formalin. Fish sample for diagnostic were found some parasite (*Lernaeasis*.,*Trichodiniasis*, *Gyrodactylus* sp. *Isopodiasis*)

**DIAGNOSIS, PATHOLOGY AND TAXONOMY OF *Perkinsus* sp. ISOLATED FROM THE
MANILA CLAM *Ruditapes philippinarum* IN KOREA**

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We report on the diagnosis, pathology, and taxonomy of *Perkinsus* sp. infection in Manila clams (*Ruditapes philippinarum*) from Korean waters. Amplimers were designed from internal portions of the non-transcribed spacer (NTS) of *P. atlanticus* for molecular diagnosis of *Perkinsus* infection. PCR-based identification methods and an *in situ* hybridization assay were developed for detection of *Perkinsus* sp. in live tissues as well as in histological preparations. Hybridization signals were observed around the nucleus of trophozoites. Positive results from PCR and *in situ* hybridization indicated that Korean *Perkinsus* sp. is genetically identical with *P. atlanticus* reported in Europe, which is currently synonymous with *P. olseni* reported from Australia. Microscopic morphological features of different life stages of *Perkinsus* sp. appeared very similar to those of *P. atlanticus*. We conclude that the *Perkinsus* sp. in Korean waters is *P. olseni* (*atlanticus*). Severely infected clams often exhibited white nodules on their mantles and gills as a consequence of inflammation. In lightly to moderately infected clams, *Perkinsus* sp. was mainly found in gill tissues, whereas the protozoan parasites were found in digestive tracts, gonadal tissues, and foot tissues of heavily infected clams. It is likely that the gills are the portal of the infection and that *P. olseni* (*atlanticus*) spreads to other tissues as the infection advances. Field surveys revealed that most clam populations of Korea's western and southern coasts (21 of 27 sampling locations) were infected to varying degrees with *P. olseni* (*atlanticus*). This indicates that *P. olseni* (*atlanticus*) is a major pathogen of clams in Korean waters

GROWTH AND MORTALITY RATES OF TWO FRESHWATER FISH SPECIES IN DYALA RIVER, NEAR BAGHDAD

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A total of 164 specimens were collected including common carp, *Cyprinus carpio* L. (78 specimens) and shilik, *Aspius vorax* (86 specimens) at the section of polluted Dyala River, Baghdad. Growth and mortality rates were described using some biological parameters during the period from February/2000 to January /2001. The regression coefficient (b) values of length –weight relationship indicated that the growths of these two species are allometric. So *C. carpio* grew less rapidly than it is length. Whereas *A. vorax* increased more than it is length. Mean condition factor (k) of *Cyprinus carpio* was 1.02 and for *Aspius vorax* was 1.08. Age group of three years for both fish species was dominated contributed 38.5% and 27.9% respectively, whereas the lowest representative groups are 4 and 5 years with 8.9% and 12.0% respectively. The ultimate growths of length (L_{∞}) were 33.9 cm and 45.7cm respectively, and for weight (W_{∞}) were 217 gm and 827 gm respectively. Cohort analysis, using Jones plot, and total mortality rate (Z) were 2.8 and 2.96 of both species respectively.

Table 1 Mean total length calculated (cm) of *C. carpio* at end of each year in Dyala river.

Age group	Mean calculated total length at each annulus(n)					Mean capture length(cm)	Mean capture weight(gm)
	n1	n2	n3	n4	n5		
1	7.9					11.3	23.0
2	6.1	12.5				15.5	56.1
3	5.8	10.8	15.8			18.2	148.9
4	5.3	10.2	15.7	20.6		22.7	150.7
5	4.1	8.5	13.8	17.9	22.8	25.0	186.6
Mean calculated length	5.8	10.5	15.1	19.2	22.8	-	-
Annual increment (cm)		4.7	4.6	4.1	3.6	-	-

Table 2 Mean total length calculated (cm) of *A. vorax* at end of each year in Dyala river.

Age group	Mean calculated total length at each annulus(n)					Mean capture length(cm)	Mean capture weight(gm)
	n1	n2	n3	n4	n5		
1	15.3					21.4	65.4
2	12.2	18.1				26.2	137.4
3	11.4	17.5	25.0			31.5	252.8
4	10.9	17.2	20.1	28.4		35.5	374.1
5	10.3	16.9	22.0	25.4	31.0	40.0	750
Mean calculated length	12.0	17.4	22.0	26.9	31.0	-	-
Annual increment (cm)		5.4	4.6	4.9	4.1	-	-

COMPARISON BETWEEN MS222 AND EUGENOL AS SEDATIVE AND ANESTHETIC AGENTS IN SHRIMP *Penaeus indicus*

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To decrease the stress-related effects of transportation and handling of shrimp, *Penaeus indicus*, application of useful anesthesia and /or sedation of animals is helpful. Two anesthetic agents, i. e. MS222 (tricaine methane sulphonate) and eugenol (4-allyl-2-methoxy-phenol), were studied using shrimp (5 ± 1 g) at 34 ± 1.7 °C. The safety range of the two anesthetic agents was established by determining the median lethal concentration (LC50). Induction of anesthesia for MS222 and eugenol was calculated as 3700 mg/l and 495 mg/l respectively. The study showed that eugenol was preferable because it showed no side effects and was cheaper (Table 1). The 48h LC50 results of eugenol was 22.5 mg/l with minimum confidence limit of 18.4 mg/l and maximum confidence limit of 27.5 mg/l (Table 2). Proper dosage of eugenol to induce sedation during 30h was estimated between 9 mg/l and 12 mg/l. Due to the narrow range between the minimum confidence limit at 48h LC50 (18.4 mg/l) and the maximum effective dosage of eugenol for induction of sedation in the experimental animal (12 mg/l), it is suggested that eugenol be used at the minimum dosage of 9 mg/l for induction of sedation, to prevent its toxic effects.

Table 1 The induction and recovery times of anesthetic properties of eugenol in *P. indicus*.

Con. of Eugenol (ppm)	Mean induction time (min.)	Mean recovery time (min.)
80	-	-
160	11.3	3.1
320	6.9	4.2
640	4.2	7.2

Table 2 The LC50, Min. and Max. confidence limit of eugenol at various exposure times for *P. indicus*.

Time (h)	Mean of LC50 (ppm)	Mean of Min. confidence limit (ppm)	Mean of Max. confidence limit (ppm)
6	52.6	46.3	60.2
12	25.2	21.5	29.3
18	23.5	19.4	28.5
24	22.5	18.4	27.5
30	22.5	18.4	27.5
36	22.5	18.4	27.5
42	22.5	18.4	27.5
48	22.5	18.4	27.5

INCREASE IN DENSITY WITH TIME OF TWO PARASITES *Trichodina* sp. AND *Cichlidogyrus* sp. INFECTING *Oreochromis niloticus* FINGERLINGS IN PACKING BAGS FOR TRANSPORT

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Low-volume high-density culture of fish favors parasite multiplication and infection of other fish. From hatcheries, fingerlings for transport to farms are usually packed densely in a transport bags. The study aimed to determine the density, in relation to time, of a protozoan parasite *Trichodina* sp. and a monogene *Cichlidogyrus* sp. infecting the fingerlings of Nile tilapia (*Oreochromis niloticus*) while in packing bags for transport to fish farms. Parasite load was also determined when the transport water medium was treated with table salt or formalin. The parasites were enumerated from 0 hr and every six hours thereafter up to 24 hr for the salt-treated and every three hours up to 12 hr for the formalin-treated while the fingerlings were in the transport plastic bags.

Results of the investigation showed that the density of the parasites, expressed as total count, prevalence and intensity, increased with time in the untreated fingerlings while the density decreased with time and with increasing concentration of salt (0 ppt, 3 ppt, 6 ppt and 10 ppt) or formalin (0 ppm, 50 ppm, 100 ppm and 150 ppm) in the water medium without apparent adverse effect on the fingerlings. The result shows that the common parasites, *Trichodina* sp and *Cichlidogyrus* sp., have the capability to multiply and infect other fish in the transport bag within the duration of transport time. It also indicates that the treatment of transport water medium with salt or formalin effectively reduces the parasite density in *O. niloticus* fingerlings. It is therefore recommended that the water medium in the transport bags should be treated with either salt or formalin to reduce the parasite load and reduce the risk of spreading the parasites.

**EPIZOOTIOLOGICAL STUDY ON MASS MORTALITIES OF THE CULTURED
SCALLOPS *Chlamys farreri* AND *Argopecten irradians***

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A systematic epizootiological study on mass mortalities of the cultured scallops *Chlamys farreri* and *Argopecten irradians* was carried out during 2000-2002. The results showed that the mortality rate of the cultivated scallop *Chlamys farreri* was very high, more than 91% from April to September, and the dead peak occurred in the second or last ten days of July; and about 60% for sea scallop *A. irradians* with the dead peak occurred in the first ten days of June. The results of investigation of environmental factors revealed that the higher water temperature and lower water temperature were the serious stress factors to *C. farreri* and *A. irradians*, respectively. In this study, several pathogens infecting the scallops were found such as Rickettsia-like organisms (RLOs), Mycoplasma-like organisms (MLOs) and virus-like particles (VLPs). It is inferred that Rickettsia-like organisms may be the main pathogen responsible for mass mortality of the cultured scallops *C. farreri* and *A. irradians* on the basis of the observation of intensity of infection and pathology.

IDENTIFICATION OF WHITE SPOT SYNDROME VIRUS (WSSV) IN *Penaeus indicus* BY POLYMERASE CHAIN REACTION (PCR) IN IRAN

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In the summer of 2002 high mortality occurred in shrimp *Penaeus indicus* in Abadan area in the south of Iran. One of the typical external signs of infected shrimp were white spot in the carapace with size 0/5-2mm. The cuticle of shrimp easily separated from the epidermis, the hepatopancreas was swollen and yellow, the intestine and abdomen was empty and the body color of infected shrimp become reddish. With high mortality and clinical signs, the shrimp suspected to White spot syndrome disease. We used polymerase chain reaction for confirming the WSSD in the shrimp. Ninety sample collected from the Abadan farm that showed the mortality and one hundred and twenty samples collected from Bousher and Hormozgan provinces and Gomishan area. The samples fixed in absolute alcohol and transport to the lab. For detection the WSSD, the sample was homogenise and DNA extraction was carried out by using phenol and chloroform methods. The WSSD kit named "Single Tube Nested DNA amplification Kit for Detection WSSD" was used for PCR. After PCR the samples from Abadan area showed 356 bp 403bp on gel agarose and the samples from other area showed 232bp. According to the Kit the samples examined in this study from Abadan area showed WSSD and the mortality occurred in the Abadan farm in Kuozestan province was due to this disease. The samples from other area were negative.

INVESTIGATION OF THE INNATE RESPONSES OF THE PRE- BROODS OF *Oncorhynchus mykiss* TO STRESS REPEATABILITY

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In fish ponds, stresses increase fish susceptibility to diseases, reduce growth and reproduction. It is believed that stress resistance can be transferred from bloodstocks to their offsprings. To find the innate responses of *Oncorhynchus mykiss* with 364 ± 60 g weigh were bled from caudal vein in two stages, i. e. after inducing stress by one hour confinement of the experimental fish and 36 hours resting after the post stress period. After labeling, the fish were kept in unstressed condition for 21 days. They were bled again in the same stages after repeating the same stress induction. The blood samples were analyzed for plasma glucose, cholesterol and total protein and compared statistically at $p < 0.05$. Based on the results of the experiment, fish were categorized into three categories according to the changes of plasma glucose (Tables 1 and 2) that was seen to be reliable compare to the other two parameters. The three groups were categorized as 1) unable to set homeostasis in setting glucose (UH) (14.7 % of the test fish), 2) able to set homeostasis in setting glucose(AH) (53.6 % of the test fish) and 3) adapted to set homeostasis in setting glucose (DH) (31.7 % of the test fish). Based on the similarity of the experimental conditions for the fish in running various stages of the test, it was suggested that a genetic factor which causes non-insulin dependent diabetes, likewise in advanced vertebrates, influences setting the fish in three categories.

Table 1: Comparison between the changes of plasma glucose (G) in the control and experimental fish after the initial (S1) and repeated phases of stress (S2) induction.

ST= Significant change NST= Not significant change

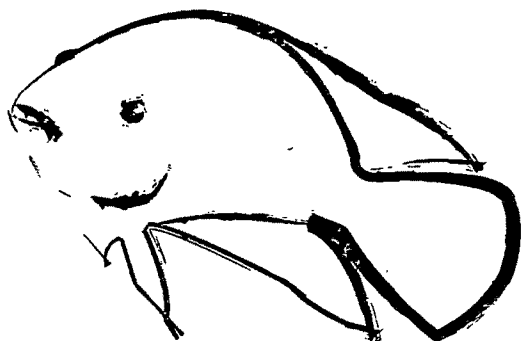
Results based on $P < 0.05$	Mean $\pm$ SD of experimental fish	Mean $\pm$ SD of control fish
ST	UHGS1 92.333 ± 23.278	SHG 72.846 ± 13.303
ST	UHGS2 117.5 ± 28.835	SHG 72.846 ± 13.303
ST	AHGS1 127.091 ± 33.115	SHG 72.846 ± 13.303
ST	AHGS2 130.273 ± 22.953	SHG 72.846 ± 13.303
ST	DHGS1 96.384 ± 16.0911	SHG 72.846 ± 13.303
ST	DHGS2 127.615 ± 20.246	SHG 72.846 ± 13.303

Table 2: Comparison between the changes of plasma glucose (G) in the unstressed and stressed fish during the initial (U1 and S1) and repeated (U2 and S2) phases of the experiment.

ST= Significant change NST= Not significant change

Results based on $P < 0.05$	Mean $\pm$ SD of plasma glucose of unstressed fish in various stages	Mean $\pm$ SD of plasma glucose of stressed fish in various stages
NST	UHGU1 105.167 ± 20.999	UHGS1 92.333 ± 23.278
NST	UHGU2 149.667 ± 42.179	UHGS2 117.5 ± 28.853
ST	AHGU1 94.272 ± 16.411	AHGS1 127.091 ± 33.115
ST	AHGU2 98.545 ± 14.637	AHGS2 130.273 ± 22.953
ST	DHGU1 130.615 ± 23.960	DHGS1 96.384 ± 16.091
ST	DHGU2 96.076 ± 14.453	DHGS2 127.615 ± 20.246

Value Added Products



AN ASSESSMENT OF TEXTURAL VARIATIONS OF MUSCLE TISSUES OF *Labeo rohita* AND *Scoliodon sorrokawah* WITH EMPHASIS ON COLLAGEN

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Structural proteins, specifically myofibrillar proteins and collagen, play a very significant role in determining the texture of the product. *Scoliodon sorrokawah* is a cartilaginous fish having high collagen content and its crude protein content (22.01 %) is comparatively higher than *Labeo rohita* (11.78 %). During freezing, an increase in the toughness was observed which was possibly due to the aggregation undergone by the myofibrillar proteins and collagen. Histograms achieved and Texture Profile Analysis data obtained using a Texture Analyzer (Lloyd Instruments, UK, model LRX PLUS) indicated a two-phase toughening process in both the species. In both fresh and frozen samples, a two to three-fold increase in toughening occurred during cooking. Though the first phase of hardening on cooking (50°C) was similar in both the species, the second phase of hardening varied in frozen samples as 70°C and 75°C for fresh and frozen-thawed shark and 70°C and 80°C for fresh and frozen-thawed rohu, respectively. These slight variations in the two species might be due to the difference in their connective tissue protein content, distribution and properties. In raw muscle tissue, the toughness is very much a product of hardening of the connective tissue proteins. During the second phase of tissue hardening, the weight loss and cooking shrinkage was observed which might be due to the loss of water holding capacity and conformational changes in the protein configuration.

Table 1 Texture Profile Analysis of *Labeo rohita* and *Scoliodon sorrokawah*.

	LABEO ROHITA					Scoliodon sorrokawah				
	Instrumental				Sensory Overall	Instrumental				Sensory Overall
Temperature	H ¹ (mm)	H ² (mm)	C	S (kgf/mm)		H ¹ (mm)	H ² (mm)	C	S (kgf/mm)	
Fresh	4.71	3.77	0.21	6.28	NA	5.13	3.87	0.13	6.60	NA
45	2.51	2.04	0.19	1.57	4	0.89	0.72	0.10	2.76	4
50	4.99	4.16	0.26	4.46	5	4.33	3.19	0.14	5.09	5
55	3.82	3.02	0.22	3.82	5	0.97	0.77	0.10	3.28	5
60	4.33	3.20	0.16	3.94	6	0.87	0.72	0.11	3.04	6
65	1.20	1.02	0.24	1.83	6	0.95	0.80	0.15	4.18	6
70	2.17	1.87	0.26	2.46	5	1.93	1.69	0.28	8.57	5
75	1.26	0.96	0.13	5.52	5	1.10	0.92	0.18	3.85	5
80	1.22	0.99	0.14	2.06	5	1.04	0.89	0.19	3.36	5
90	2.16	1.92	0.29	2.47	5	2.04	1.76	0.29	2.94	5
Frozen	5.12	3.88	0.14	6.34	NA	4.33	3.53	0.20	5.45	NA
F-45	4.61	3.49	0.12	4.89	4	5.42	4.40	0.22	5.64	4
F-50	5.86	4.54	0.19	6.05	4	6.41	5.08	0.23	6.81	4
F-55	3.27	2.65	0.17	3.04	4	2.42	1.97	0.18	3.55	4
F-60	2.02	1.53	0.13	3.82	4	1.56	1.22	0.14	1.68	4
F-65	2.14	1.68	0.12	3.69	5	1.51	1.18	0.15	1.29	5
F-70	1.61	1.27	0.14	2.37	5	1.65	1.31	0.16	1.93	5
F-75	1.37	1.09	0.12	2.74	5	1.75	1.35	0.18	3.95	5
F-80	1.26	0.95	0.12	5.87	5	1.37	1.08	0.14	1.26	5
F-90	1.45	1.18	0.18	2.38	5	1.53	1.24	0.16	1.80	5

IMPROVEMENT OF QUALITIES OF TRADITIONAL MARINE DRIED PRODUCTS USING A LOW COST SOLAR DRIER

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A comparative study was conducted on the nutritional and food qualities of some commercially important traditional dried marine fish products available in the market and the dried products produced in a low cost solar tunnel drier by determining organoleptic, biochemical and bacteriological aspects. Detailed survey of various marketing chains revealed that considerable post-harvest losses occur both qualitative and quantitatively through spoilage and insect infestation which account for 10-35% of the total dry fish production. Organoleptically the products obtained from the retail markets were in poor quality of rancid and objectionable odour compared to those obtained from wholesale and producer level. On the other hand, the products produced in the solar drier were excellent quality in terms of colour, odour and texture. The results on the chemical composition and food qualities of raw material traditionally dried and solar dried products of five marine fish species are given in the table 1 below. The study revealed that moisture content, peroxide value, total volatile base and aerobic plate counts in traditional dried products available in the markets were much higher than those produced in the solar drier. Besides, there was widespread use of insecticides such as nogos and basudin in the traditional dried products in all marketing chains. The shelflife study of the raw materials indicated that all the five fish species immediately after harvest were found in acceptable condition for 10-12 days in ice storage. The study also revealed that all the fish species after 10-12 days of ice storage can effectively be utilized as raw material for the production of high quality dried products in a low cost solar drier and these dried products were in acceptable qualities for 8-10 months in various packaging conditions.

Table 1 Nutritional and food qualities of traditional and solar dried marine fish products of Bangladesh

Quality characteristics	Raw material	Traditional	Solar dried
Chemical composition			
Moisture	77.10 – 88.51%	17.80 – 31.42%	14.48 – 16.68%
Crude Protein	10.02 – 19.11%	42.51 – 58.93%	58.13 – 68.49%
	(64.60 – 87.2 %)	(60.60 – 73.04%)	(70.34 – 78.72)
Crude Lipid	1.04 – 3.02 %	1.97 – 9.80%	5.63 – 10.08%
	(5.03 – 14.81)	(2.80 – 11.34%)	(6.92 – 11.58%)
Ash	1.07 – 5.17%	16.95 – 26.84%	11.95 – 16.58%
	(8.90 – 25.03)	(21.17 – 26.84%)	(13.73 – 20.39)
Food quality			
Organoleptic characteristics	Characteristics of fresh fish	Poor quality due to spoilage, broken and insect infestation	Characteristics of high quality, no insect infestation
Reconstitution behavior	—	at 80°C 65% max.	at 80°C 85% max
Peroxide value	3.10 – 9.51	12.56 – 64.84	12.43 – 26.44
TVB-N	22.84 – 32.05 mg/100 g	15.24 – 39.13 mg/100g	19.85 – 27.98 mg/100g
	—	Nogos, basudin	Insecticide free
Use of insecticide	2.41 x 10 ³ –3.92x10 ⁴	1.72 x 10 ⁴ –1.60x10 ⁵	1.88 x 10 ³ –3.60x10 ⁴
Aerobic plate count	CFU/g	CFU/g	CFU/g

Parentheses indicates the dry weight basis

A PRELIMINARY STUDY ON THE AMINO ACID AND NUCLEOTIDE PROFILE IN TILAPIA *Oreochromis niloticus* WASTE

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The total aquaculture production of tilapia in Malaysia was about 18,277 metric tons valued at RM 95.30 million as compared to only 8,798 metric tons in 1995. The production is expected to increase up to 40,000 metric tons valued at RM 200 million by year 2010. Processing of fish usually leads to enormous generation of waste. More than 20,000 metric tons of waste is therefore expected to be generated in the next 5 years. In fish, more than 90% of the extracted nitrogen compounds can be accounted for by free and combined amino acids, nucleotides and related substances. The objective of this study was to evaluate the amino acids and nucleotides in tilapia waste to explore the possibilities of their utilization.

Tilapia waste (head, bone, skin & viscera) and tilapia muscle were freeze dried before analyzing with HPLC to determine the amino acids and nucleotides content. Glutamic acid, aspartic acid, glycine and alanine were amino acids that were found to be predominant in tilapia. These amino acids were important in contributing to flavour especially glutamic and aspartic acids. Glutamic acid and aspartic acid were observed to be higher in tilapia muscle compared to tilapia waste. However, glycine and alanine were found to be higher in tilapia waste compared to tilapia muscle. Further exploration in evaluating the potential of tilapia waste is important in order to reduce the waste problem in the future.

Inosine 5'-monophosphate (IMP) and adenosine 5'-monophosphate (AMP) are important in flavor ingredients. The high content of IMP in tilapia muscle indicated that ATP-catabolism occur rapidly in fish muscle. AMP-deamination does not occur rapidly in tilapia waste therefore AMP was found to be higher in waste than in muscle.

Table 1 Main amino acids and nucleotides in tilapia waste that contribute to flavor (expected waste per 100 g dry sample is 70 g)

	Muscle (g/ 30 g dry sample)	Waste (g/ 70 g dry sample)				Total waste
		Head	Bone	Skin	Viscera	
Amino acid						
Glutamic acid	5.29	4.91	5.13	6.80	3.45	5.07
Aspartic acid	3.11	2.97	3.08	3.89	2.23	3.04
Glycine	1.76	4.21	4.71	9.28	1.40	4.90
Alanine	2.38	3.02	3.14	5.12	1.79	3.27
Nucleotide						
IMP	54.31	5.36	3.65	5.21	12.33	6.64
AMP	3.74	35.58	45.08	42.65	11.46	33.69

FACTORS AFFECTING THE NUTRITIONAL COMPOSITION OF SNAKEHEAD *Channa punctatus*

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Proximate composition of snakehead, *Channa punctatus*, was determined from fish of various sizes (small, medium and large) and sexes (male and female), from different body parts (head, middle, tails and eggs), and from fish processed differently (raw flesh, sun drying, oven drying, boiling, and freezing). The values for protein and lipid were gradually increased from small to large size fish while ash and moisture contents gradually decreased from small to large fish (Table 1). For different body parts, maximum protein, lipid, and moisture contents were observed in middle parts of the body and maximum ash was observed in head region (Table 2). Though the eggs contained almost the same level of protein as for other body parts, lipid excelled considerably than any other parts of the body. The protein and lipid contents were high in male where as ash and moisture contents were high in female fish (Table 2).

Proximate composition varied widely depending on the various processing methods (Table 3). The protein contents of fish receiving different processing treatments – raw flesh, sun drying, oven drying, boiling, frying and freezing were $19.71 \pm 0.02\%$, $59.34 \pm 0.69\%$, $66.52 \pm 4.87\%$, $19.59 \pm 0.67\%$, $22.97 \pm 0.38\%$, and $21.11 \pm 0.32\%$, respectively. Percentage of lipid content ranged from $0.65 \pm 0.43\%$ to $3.23 \pm 0.23\%$ with the highest in sun drying and the lowest in boiling fish. On the other hand, ash contents varied from $1.21 \pm 0.15\%$ to $34.18 \pm 1.29\%$. Maximum ash was found in oven drying and minimum in boiled fish. The present study is comprehensive in nature and can be used as reference in the formulation of feeds, and to determine the health and nutritional status of commercially reared snakehead.

Table 1 Nutritional composition of different size classes of *C. punctatus*.

Size class	Size range	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Small size	7-9 cm	17.77 ± 0.14	0.73 ± 0.14	2.45 ± 0.39	82.73 ± 1.03
Medium size	9-11 cm	18.64 ± 0.18	1.06 ± 0.18	2.16 ± 0.17	80.84 ± 0.04
Large size	≥ 11 cm	19.37 ± 0.21	1.10 ± 0.03	1.75 ± 0.02	79.64 ± 0.10

Table 2 Nutritional composition of different body parts of *C. punctatus*.

Sex	Parts	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Male	Head	19.86 ± 0.02	1.41 ± 0.07	2.55 ± 0.01	76.31 ± 0.09
	Middle	20.69 ± 0.89	2.30 ± 0.23	1.61 ± 0.72	77.43 ± 0.89
	Tail	19.37 ± 0.07	2.17 ± 0.01	1.25 ± 0.01	76.95 ± 0.12
Female	Head	18.81 ± 0.56	1.32 ± 0.02	3.54 ± 1.21	78.02 ± 0.01
	Middle	20.35 ± 0.04	1.78 ± 0.19	1.47 ± 0.75	78.08 ± 0.19
	Tail	17.35 ± 0.94	1.34 ± 0.15	1.80 ± 0.19	77.67 ± 0.71
	Fish egg	18.63 ± 1.29	30.03 ± 1.40	1.60 ± 0.08	Not determined

Table 3 Nutritional composition of *C. punctatus* with varying conditions.

Conditions	Protein (%)	Lipid (%)	Ash (%)
Raw flesh	19.71 ± 0.02	1.10 ± 0.007	1.75 ± 0.009
Sun drying (3 d)	59.34 ± 0.69	3.22 ± 0.23	4.74 ± 0.61
Oven drying (24 h)	66.52 ± 4.87	1.23 ± 0.19	34.18 ± 1.29
Boiling	19.59 ± 0.67	0.65 ± 0.43	1.21 ± 0.15
Frying	22.97 ± 0.38	1.55 ± 0.31	1.52 ± 0.02
Freezing	21.11 ± 0.32	1.26 ± 0.40	1.46 ± 0.08

MONTHLY VARIATION IN THE BODY COMPOSITION OF SNAKEHEAD *Channa punctatus* (BLOCH, 1974)

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Annual changes in nutrient content like protein, lipid, ash, and moisture were assessed in snakehead, *Channa punctatus*, in both sexes during May 2002 to April 2003. In case of male, percentage of protein content varied from 16.78 ± 1.27 to $22.34 \pm 0.31\%$ with maximum content in November and minimum in July. The highest lipid content was observed in February ($2.49 \pm 0.18\%$) and minimum in July ($0.76 \pm 0.33\%$). The average value of ash content ranged from 1.33 ± 0.02 to $2.13 \pm 0.58\%$ with maximum values in September and minimum in August. The percentage of moisture content in the present study ranged from 76.57 ± 0.18 to $81.34 \pm 1.36\%$ having maximum values in August and minimum in November. On the other hand, in case of female fish, the highest value of protein ($22.58 \pm 2.02\%$), lipid ($2.26 \pm 0.32\%$), ash ($2.29 \pm 0.18\%$), and moisture ($80.01 \pm 0.15\%$) was found in the month of September, February, April, and August, respectively, while the lowest value of protein ($17.85 \pm 2.70\%$), lipid ($0.91 \pm 0.08\%$), ash ($1.16 \pm 0.12\%$), and moisture ($77.04 \pm 1.23\%$) was found in the month of June, November, December, and February, respectively. The present findings suggest that the variation of body constitutes could be due to the influences of season, age, hydrological parameters, diet, as well as the maturation, gravid and spent stage of fish. From the nutritional point of view it is evident that the biochemical composition of *C. punctatus* is almost similar to that of other small valued species and even in large fishes.

Table 1. Monthly variation of body composition (male and female) of *C. punctatus*.

Sex	Months	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Male	May	19.97 ± 0.54	1.97 ± 0.40	1.47 ± 0.15	76.90 ± 0.45
	June	18.61 ± 0.41	1.19 ± 0.05	1.76 ± 0.16	79.94 ± 0.32
	July	16.78 ± 1.27	0.76 ± 0.33	1.45 ± 0.29	80.08 ± 0.68
	August	19.02 ± 0.06	1.55 ± 0.03	1.33 ± 0.02	81.34 ± 1.36
	September	19.47 ± 0.61	1.09 ± 0.02	2.13 ± 0.58	78.52 ± 2.50
	October	20.84 ± 0.20	1.33 ± 0.15	1.57 ± 0.03	79.43 ± 0.10
	November	22.34 ± 0.31	2.08 ± 0.28	1.72 ± 0.01	76.57 ± 0.18
	December	21.46 ± 0.94	1.67 ± 0.45	1.33 ± 0.15	77.67 ± 0.94
	January	19.69 ± 0.20	1.07 ± 0.03	1.51 ± 0.24	80.80 ± 0.47
	February	22.18 ± 0.15	2.49 ± 0.18	1.49 ± 0.07	76.71 ± 0.03
	March	18.79 ± 0.02	1.08 ± 0.14	1.49 ± 0.02	78.47 ± 0.29
	April	17.89 ± 1.73	1.35 ± 0.08	1.78 ± 0.05	79.60 ± 0.51
Sex	Months	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Female	May	18.84 ± 1.22	1.48 ± 0.21	2.27 ± 0.90	77.92 ± 0.18
	June	17.85 ± 2.70	0.92 ± 0.14	1.93 ± 0.01	79.78 ± 0.12
	July	21.01 ± 2.38	1.13 ± 0.18	1.48 ± 0.03	79.51 ± 0.40
	August	18.45 ± 1.24	1.10 ± 0.28	1.55 ± 0.39	80.01 ± 0.15
	September	22.58 ± 2.02	1.08 ± 0.05	1.67 ± 0.07	79.17 ± 0.92
	October	20.32 ± 1.33	1.61 ± 0.29	1.61 ± 0.20	79.96 ± 1.22
	November	20.36 ± 0.19	0.91 ± 0.08	1.73 ± 0.04	79.31 ± 0.25
	December	20.48 ± 1.55	2.01 ± 0.05	1.16 ± 0.12	78.25 ± 0.91
	January	18.78 ± 1.69	1.05 ± 0.26	1.59 ± 0.13	79.03 ± 0.47
	February	22.21 ± 0.67	2.26 ± 0.32	2.28 ± 1.04	77.04 ± 1.23
	March	20.55 ± 0.81	1.65 ± 0.47	1.51 ± 0.20	79.13 ± 1.50
	April	18.008 ± 1.27	1.47 ± 0.01	2.29 ± 0.18	77.92 ± 0.15

BIOCHEMICAL EVALUATION OF *Pangasius hypothalamus* STORED IN ICE AND AT AMBIENT TEMPERATURE

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An investigation was carried out to evaluate the biochemical quality of Pangus (*Pangasius hypothalamus*) stored in ice and at ambient temperature (23-25°C). Pangus is one of the increasingly important species, along with market demand, for aquaculture in Bangladesh. During storage in ice and at ambient temperature, fish underwent degradation. The use of TVB-N, TMA-N and pH as a criterion for fish freshness with respect to *Pangasius hypothalamus* were evaluated. The nature of biochemical changes was studied during iced storage over a period of 17 days and at ambient temperature for 24 hours. Live fish were collected from local ponds. Fish were kept in insulated boxes with crushed ice at a ratio of 1:1 and in bamboo basket covered with polythene paper at ambient temperature. In iced storage study, the value of TVB-N, TMA-N and pH ranged between 23.29 ± 0.18 to 33.15 ± 1.63 , 5.47 ± 0.24 to 8.69 ± 1.88 and 6.95 ± 0.13 to 7.0 ± 0.03 , respectively, when fish were in acceptable limit during 11-14 days. At ambient temperature, the muscle TVB-N, TMA-N and pH values were 44.03 ± 1.38 , 5.65 ± 0.13 and 6.69 ± 0.02 , respectively, at rejection time (23h). The results obtained showed that all values increased progressively as spoilage advanced. The TVB-N and TMA-N values were found to have lower values on 3rd day of iced storage. All values determined were co-related with storage time under both storage conditions. Significant statistical differences ($P \leq 0.05$) between storage time and values were found under both storage environments. The comparative study indicated that fish spoiled much faster at ambient temperature. TVB-N value and pH could be used as spoilage indicator. However, TMA-N values were not found to be a suitable indicator for the species studied under both iced storage and ambient condition.

**EFFECT OF DIFFERENT HANDLING PRACTICES ON THE QUALITY OF ICE- STORED
TILAPIA *Oreochromis mossambicus* USING QUALITY INDEX METHOD (QIM)**

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Tilapia production in Sri Lanka has increased during the recent past. The international trade in tilapia is growing steadily. It is important to develop suitable techniques for killing and handling and also the improved storage facilities to retain freshness. Quality Index Method (QIM) has provided the industry with a practical and powerful tool for measuring the freshness of fish. QIM is based on significant sensory parameters and score system from 0-4 demerit points. The theoretical demerit curve is fixed at the point (0, 0) and its maximum has to be fixed where the fish stored in ice is rejected by sensory evaluation. The focus of this study has been on development of QIM for tilapia (*Oreochromis mossambicus*) by utilizing different killing methods. The outcome of this study will be of immense use for fish auction centres and also for quality management in tilapia processing plants.

The specimens of tilapia (*O. mossambicus*) were collected from Thabbowa reservoir using cast nets and were brought to the landing centre without killing the fish. Tilapia were divided into four equal groups and two groups were killed instantly. Of the instantly killed fish, one group was kept without gutting (IK) while the remaining fish group was gutted (IKG). Two remaining groups were allowed to die normally and followed the similar procedure as the instantly killed fish i.e. gutted (NG) and ungutted (N). Each group was kept in polythene bags and stored in ice during transportation to the laboratory.

The tilapia stored in ice at 0°C scored maximum demerit points of 20 for 25, 30, 35 and 40 days of storage for different killing and handling practices N, IK, NG and IKG, respectively. The different groups of fish N, IK, NG and IKG stored in ice scored 10 demerit points after storage of 12, 15, 18 and 25 days, respectively.

The study revealed that the storage time of sealed tilapia in ice could be increased by 60%, if the IKG method is used when compared to the normal death procedure (N). The estimated protein and oil content of tilapia when it scores 10 demerit points during IKG method were 18.13 (90%) and 1.36 (93%) respectively from initial values. The IKG and NG method of killing fish were found to be very much appropriate for tilapia for long term storage in ice.

PROCESS ACCOUNTING APPLICATION TO SHRIMP PROCESSING

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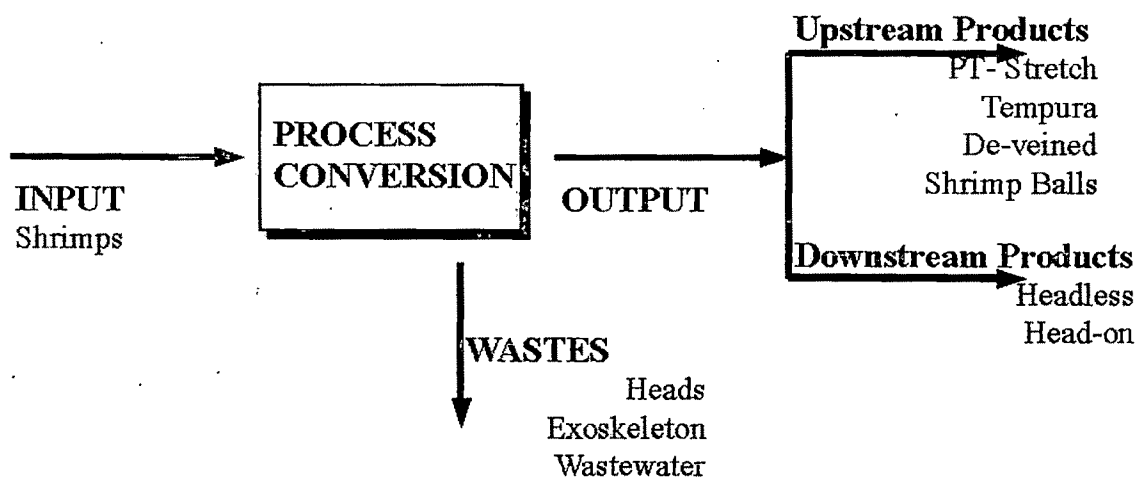
Process Accounting is a method that defines the appropriate process streams in the conversion of raw materials to products, with the end result of:

- Increasing utility of the raw materials;
- Minimizing losses as wastes; and
- Implicitly mitigating and protecting the environment.

Black tiger shrimp (*Penaus monodon*) is a major cultured specie in the Philippines. Its normal markets are fresh/chilled, and at times minimally processed. When minimally processed, product forms vary from frozen whole, to frozen headless, and to value added products: peeled and de-veined, pt - stretch, tempura, and shrimp balls, to name a few. The shrimp heads and exoskeleton are often generated as wastes. These shrimp solid wastes could serve as raw materials for bioactive compounds; the carotenoids, and chitosan. Carotenoids are fat soluble pigments, and chitosan is a polysaccharide with a lot of functional attributes.

The paper discusses the applications of process accounting to the processing of shrimps. Process accounting, as a management tool, is being advocated as a protocol in the design of seafood processing operations.

Figure 1 Process accounting applications to shrimp processing



LACTIC ACID TREATMENT OF REFRIGERATED TILAPIA FILLETS

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Tilapia is a popular fish among the Chinese cuisine. It is marketed as whole fish in majority of the cold chain stores. However, due to the present life style, the availability of the fish in its fillet form is an added advantage to the customer as well as the retailer. The shelf life and the pattern of spoilage of fillets vary. Lactic acid dipping at four different contact times was evaluated in this study for the purpose of determining its effectiveness in enhancing the shelf life of these fillets. Live tilapia was brought to the laboratory, filleted, washed under running tap water prior to the acid dipping. They were then individually packed in polystyrene trays with an overwrap and stored at 4 °C. It was noted that the 3 % lactic acid dip was able to reduce the production of total volatile base nitrogen (TVBN). The treatment was also able to reduce the rancidity development (TBARS analysis) in the fillets. Although there were differences in absolute values between treatment and control, the trend of change of these two indicators were similar. The sensory evaluation carried out indicated that the treatment had caused a detectable change in the fillets. Treated and stored fillets were evaluated for overall appearance and odor development by untrained panelists. Initially, the treated tilapia was scored lower for overall appearance, which could be due to the surface changes of the fillets. It was concluded that the surface change is one of the detrimental effects of acid treatment on the quality characteristics of the fillets (Table 1). Similar scores were also obtained for odor evaluation. It was commented that the treated fillets had detectable acidic odour. Based on the scores given, the control fillets had an acceptable odour of up to 3 days and those of treated fillets up to 6 days. However, the chemical scores for treated samples are acceptable even up to day 15, i.e. the last day of the storage study period. The low sensory scores for all samples both control and treated could be due to the presence of the drip in the packages. It was also concluded that the contact time suitable for an effective treatment should be in the range of 60 to 120 sec.

Table 1 Mean appearance and odour scores of control (15 sec) of tilapia fillets during storage at 4°C

Treatment	Storage time (days)					
	0	3	6	9	12	15
¹ Control 0.43 ^{CB} (15 sec)	8.6 ± 0.44 ^{aA}	6.6 ± 0.42 ^{bA}	5.2 ± 0.40 ^{cA}	3.1 ± 0.56 ^{dA}	2.7 ± 0.33 ^{eA}	2.0 ±
² Control 0.31 ^{FA} (15 sec)	8.5 ± 0.66 ^{aA}	6.5 ± 0.26 ^{bA}	3.1 ± 0.41 ^{cA}	2.1 ± 0.38 ^{dA}	1.4 ± 0.92 ^{eA}	1.0 ±

1-appearance, 2- odour

APPLICATION OF QUALITY INDEX METHOD IN DETERMINING THE SHELF LIFE OF SKIPJACK TUNA *Katsuwonus pelamis*

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The freshness of fish is the most concerned quality attribute by the consumer and the freshness of fish deteriorates with time, until the product is no longer acceptable for human consumption. The main aim of this study was to develop and evaluate a Quality Index Method (QIM) scheme, one of freshness quality grading systems, for skipjack tuna (*Katsuwonus pelamis*). The QIM provides weighted evaluation of the key parameters in deterioration of individual species, assigning demerit points according to the importance of each parameter. For the development and evaluation of QIM scheme, skipjack tuna were stored in ice and assessed raw and cooked. The QIM developed for raw skipjack tuna consists of 11 parameters (appearance of skin, stiffness; cornea, form and color of pupil of eye; color, smell and mucus of gills; condition of viscera; color of blood; and color of fillets) covering attributes, which gave a total 25 demerit points. Sensory analysis of cooked skipjack tuna using the Torry scheme were carried out in parallel to determine the shelf life. In order to gain further information about the quality of skipjack tuna, Total Viable Counts (TVC), hydrogen sulphide producing bacteria (spoilage bacteria), Trimethylamine (TMA), Total Volatile Nitrogen (TVN) content, and Histamin content were determined.

The sensory evaluation was carried out by a panel of 7 members and the rejection level was found to be 20 days. The QIM scheme developed for skipjack tuna showed a linear relationship between QIM scores and storage time in ice, ($r^2=0.9827$) with slope of 0.9279. The results from sensory analysis of cooked skipjack tuna indicated that 20 days was the maximum storage time in ice.

The microbial counts increased exponentially with storage time and total bacterial counts varied from 10^2 cfu/g to 10^5 cfu/g within 2 to 20 days and spoilage bacteria varied from 10^2 cfu/g to 10^4 cfu/g within 6 to 20 days of storage in ice. The TMA and TVN amount is increased with time and the amount were 1.14 mg/100g to 5.26 mg/100g and 7.53 mg/100g to 34.20 mg/100g within 2 days to 20 days in ice. Histamin content ranged from 9.5 mg/100g to 148.6 mg/100g within storage. The variation of pH was from 5.53 to 5.76 with the time of storage in ice.

The QIM calibration curve developed for skipjack tuna indicates its applicability to determine the storage life of fish in ice. Further, it will facilitate the requirements of buyers and sellers while fulfilling the demands of inspection authorities and the consumers.

HACCP & AQUACULTURE

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The current definition of aquaculture is the farming of aquatic organism including fish, crustaceans and aquatic plants that most of them are used by human directly or indirectly. Hazard analysis critical control point or HACCP is a food safety management system which concentrates prevention strategies on known hazards and the risks of the occurring at specific points in the food chain from farm to fork. Combination aquaculture activities and HACCP system and application of them together in aquaculture is a best way to perform suitable processes and making sure the clients and consumers about your products safety.

In this paper I have tried to introduce monitoring system about residues surveillance plan have carried out according to my country requirements that were approved and issued (the requirements of council directive 96/23/EC) by veterinary organization as a competent authority in my country. This programme only covers shrimp and contains selection of samples, substances groups which should be monitored in aquaculture and their products, sampling procedures and responsibilities and coordination.

Iran's inland intensive and semi-intensive shrimp aquaculture industry extends over 3635 hectares in 2002. The main system of shrimp culture is in earth ponds. In the case of shrimp production, the principal source of water is seawater while in some of them freshwater is used to balance the salinity. The most commonly cultivated shrimp species is Indian white shrimp (*Penaeus indicus*) although some others such as *penaeus semisulcalus* and *penaeus merguensis* are also cultured. The mean annual aquaculture production for 2003 in Iran was approximately 6800 tons.

To comply with the annex IV of 96/23 directive requirement of one sample per 100 tones of annual production it was necessary to take at least 68 samples for analysis. A second criterion was that samples should be collected from a minimum of 10% of registered sites of production. The total number of registered farms at that time was 244; therefore at least 47 samples were required. Since it was third year of implementation of this programmed and in 2003 total number of samples were collected 47. These samples were divided to tow groups as follows:

- 15 samples (31.9% of samples) were checked for group A substances included substances having anabolic effects and unauthorized substances such as Stilbenes and their derivatives, and their salts and esters, steroids. Also compounds included in Annex IV to Council Regulation (EEC) No 2377/90 of 26 June 1990.
- 32 samples (68.1% of the samples) were checked for group B included veterinary drugs and contaminants as antibacterial substances, other veterinary drugs and Anthelmintics, Other substances and environmental contaminants, organochlorine compounds including PCBs, Chemical elements, Mycotoxins, Dyes.

AFSSA Fougères, the French national reference laboratory, were designated as the Reference Laboratory (RL) for the analysis and testing of Chloromphenicol and other compounds, antibacterial substances and Dyes and Malachite green. AFSSA Paris the French national reference laboratory was designated as the Reference Laboratory (RL) for the analysis and testing of organochlorine compounds including PCBs- B3a and heavy metals.

According to the tests results the residues of organochlorine compounds (PCBs and Anti-biotic residues, Mycotoxins and etc) were negative and the mean of lead was 43.1 µg/kg and mercury was 15.8 µg/kg. That means the result of all tests were acceptable and in confirmatory levels which were verified by competent authority.

**PIGMENT EXTRACTION FROM *Acipenser guldenstadti* AND *Acipenser persicus*
CAVIAR BY HPLC METHOD**

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Due to importance of the color for caviar quality and its export, in this project it has been attempted to identify elements which yield caviar color.

In this respect, some samples of caviar grade 1A and 1B were taken from *A. guldenstadti* and *A. persicus* species and a complementary sample of *A. stellatus* Species grade 2, the caviar pigment amounts were measured through HPLC method.

To do this, the required materials were collected simultaneously; Some Samples were taken from southern coast of the Caspian Sea in Golestan Province were taken to food Industry institute Laboratory in Tehran Subjected to Axote gas and under darkness. Only a little amount of *A. Guldenstadti* Caviar grade 1A sample was gained from cold Storage of Shilat Trading Corp.

The tests are done with standards of the pigments with high purity –Such as B- Carotene Lutein, Astaxantin, etc. The chemical Substances and solvents used were obtained from merk and Fluka Companies.

Therefore it was Stated that color variety among avarious caviear grads has a meaningful relationship with the quantities of these pigments in various types of caviar. The quantity of B-caroten, Astaxantin and Lutein Pigments were found to be more in *A. Guldenstadti* caviar than in *A. persicus* Caviar, and similarly they are more in caviar 1 A than in 1B in *A. persicus* and *A. Guldenstadti* species.

As a result, it could be said that there is a significant relation ship between the amount of the pigments and the light yellowish color of the distinct caviar samples analyzed.

MICROBIOLOGICAL QUALITY OF SHRIMP *Penaeus monodon* IN RELATION TO PHYSICO-CHEMICAL FACTORS IN WATER SOURCES

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Culturing of shrimp, *Penaeus monodon* in natural waters make them more prone to contamination by pathogenic microorganisms. The prevalence of the pathogenic bacteria, *Salmonella*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, total coliforms, faecal coliforms and *Escherichia coli* in water samples from water sources (Dutch canal and Mundal lake) for shrimp farming were determined. Salinity, pH and suspended solids in water were estimated. The impact of pH and salinity on the growth of pure isolates of *V. parahaemolyticus*, *E. coli* and *Salmonella* were examined. The combined effect of salinity and pH on the survival of *Salmonella* spp., *E. coli* and *V. parahaemolyticus* was determined.

Water samples were collected from six sampling points. The total coliform count of water varied between 11 to 1800 MPN/100ml. The faecal coliforms and *E. coli* were between 2 - 175 MPN/100ml and 0-95 MPN/100ml respectively. *Salmonella arizonae* was observed in one occasion while *V. parahaemolyticus* observed in two occasions at the population of 10¹ cfu/g. The physicochemical parameters varied among sampling points monitored. Total coliforms, faecal coliforms and *E. coli* were isolated from waters at salinities ranging from 8 to 37 ppt with the majority of samples from 14 to 28 ppt range. Total coliforms, faecal coliforms and *E. coli* were observed mostly in water samples with pH values and suspended solid levels ranging from 7.9 to 10.0 and 50 to 98 mg/l respectively. The highest population of total coliforms was recorded in water with low salinity (8 ppt) and pH (8.2).

Growth of *V. parahaemolyticus*, *Salmonella* and *E. coli* was observed at 5–100 ppt, 5–40 ppt and 2–65 ppt salinity levels and pH range of 3.0–10.5, 3.5–9.8 and 3.5–10.3 respectively under experimental conditions.

There was no growth of *E. coli* when the salinities were 35 and 40 ppt and pH values were 9.0 and 9.5. The least growth of *Salmonella* spp. was observed when the combination of salinity level was 40 ppt and pH values were 9.0 and 9.5. Higher growth of *V. parahaemolyticus* has been observed at all combinations of salinities ranging from 15 to 40 ppt and pH 7.5–9.5. The growth was optimum at salinity level of 40 ppt and pH 7.5–9.5.

Physico-chemical parameters of water used for shrimp farming in Dutch canal and Mundal lake provide favourable conditions for the growth of *Salmonella* spp., *V. parahaemolyticus*, Coliforms, Faecal coliforms and *E. coli*.

IMPLEMENTING TQM IN SEAFOOD INDUSTRY IN KERALA, INDIA

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It has nearly been a decade now since the implementation of HACCP by the seafood industry in India. This paper aims at perceiving the gap between the present scenario of quality management in the seafood industry in Kerala, India, and the future prospect of developing Total Quality Management (TQM). TQM is an integrative philosophy of management for continuously improving product and process quality to achieve customer satisfaction. The gap analysis is done on the basis of ten constructs of TQM implementation, namely, top management commitment, customer focus, supplier quality management, design quality management, benchmarking, Statistical Process Control (SPC) usage, internal quality information usage, employee involvement, employee training and employee empowerment. An attempt is also made to study the costs involved and the benefits accrued in the process.

Sampling survey technique with survey instruments of questionnaires and interviews was used to obtain data. Random sampling technique was used to select the plants under study. As cochin is the centre of genesis of seafood export from India, the focus of attention was devoted to it. Five plants were taken for in-depth study, while almost 80% of the eu plants in cochin were sampled generally.

The findings suggested the importance of development of a suitable two-pronged strategy, including both HACCP and TQM for successful implementation in the seafood industry, designed to meet its specific requirements. The analysis indicated that TQM implementation leads to better product quality. It was seen that the implementation of HACCP by all firms in the industry, helped to a great extent in refining constructs like top management commitment, customer focus, design quality management and employee training, vis-à-vis its suitability for TQM implementation. The perceived gap was found to be greater with regard to the constructs like supplier quality management, benchmarking and SPC usage, than for the others. The findings also revealed that size of the firm was not a deterrent to the usage of TQM system.

An assessment of the above will be useful in future studies for analyzing the degree of resource commitment needed in order to successfully implement TQM.

FACTORIAL STUDY OF VARIOUS CHEMICALS AND NaCl EFFECTS ON THE GROWTH AND TOXIGENESIS OF *C. botulinum* AND *E.coli* IN A BHI BROTH MODEL EXPECTED FOR CAVIAR FORMULATION

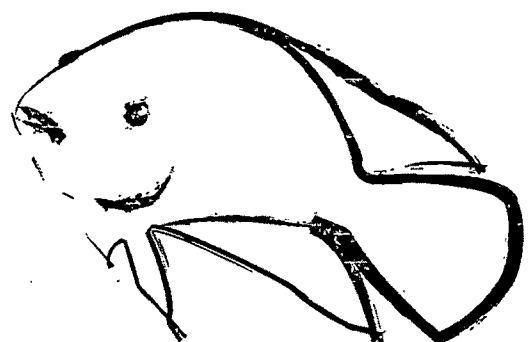
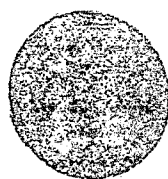
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To evaluate a new formulation of preservatives in caviar, a total of 16 formulations of NaCl and chemicals were used as a preliminary study in a broth model. These formulations contained 3 concentrations of salt (4.5, 5 and 5.5%) and 4 combinations of preservatives including (1) a mixture of 0.3% boric acid with 0.4% of borax, (2) a mixture of 2.6% K-sorbate with 80 ppm of Na-nitrate, (3) .1% of methyl paraben and (4) 0.2% of methyl paraben. All of these formula were made in Brain Hearth Infusion (BHI) broth and inoculated with 2 bacteria (*C. botulinum* type E spores and *E. coli* O₁₁₁ cells) using $>10^5$ spores or cells per ml of broth and stored up to 7 days at 30° C. *Clostridium botulinum* was examined for both growth and toxicity testing using bioassay technique. The results indicated that the lowest antimicrobial activity belonged to the combination of boric acid and borax plus NaCl concentration of 4.5 to 5.5% with only bacteriostatic activity. The highest antimicrobial activity belonged to the combination of 0.1 to 0.2% methyl paraben plus concentration of 4.5 to 5.5% with a strong bacteriocidic activity. Increasing salt from 4.5 to 5.5% concentration slightly increased the anticrobial activity of the additive combination. Therefore methyl paraben, which is a GRAS (Generally Recognized As Safe) preservative, and has a strong antimicrobial activity at high pH values, was selected for use in caviar.

***Enhancing the Ornamental
Fish Industry***



**EVALUATION OF POLYMERASE CHAIN REACTION (PCR) FOR DETECTION OF KOI
HERPES VIRUS (KHV) FROM COMMON CARP AND KOI IN INDONESIA**

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Koi herpes virus (KHV) is recognized as the cause of mass mortality in population of common carp *Cyprinus carpio carpio* and fancy carp or koi *Cyprinus carpio koi* in Israel and the USA in 1998. Cases with similar symptoms also have been occurring in Indonesia throughout Java Island since March 2002. High mortality (80-90%) in both common carp and koi carp was observed. Indonesia has not experienced a disease of this nature in the past. A polymerase chain reaction (PCR) assay was developed to detect the presence of the viral agent in gill, liver, spleen, kidney and muscle of infected fish. The specific primers were developed to amplify a 400 base pair (bp) DNA sequence from KHV, (GeneBank Accession no. AF411803 and AB127966) and a 205 bp DNA sequence for β -actin gene as internal control (GeneBank Accession no. M24113). Assay sensitivity was determined using as little as 1 fg of KHV DNA plasmid in the presence of 200 ng of uninfected fish genomic DNA. The results suggested that this method could be used for rapid and sensitive detection of KHV to support histological examination. We are currently applying this system for quantitative (real-time) (using SYBR Green) PCR.

FRESH WATER ORNAMENTAL FISHES OF KERALA, INDIA, THEIR POTENTIAL AND CAPTIVE SURVIVAL IN AQUARIA

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The diverse inland water bodies of Kerala are blessed with a variety of untapped potential ornamental fish resources. The fresh water ornamental fish fauna of Kerala is represented by 125 species, majority of them suitable for keeping in home aquaria due to their gorgeous colouration and hardy nature. The indigenous fresh water ornamental fish resources in Kerala include - *Puntius denisonii*, *P. sophore*, *P. jerdoni*, *Tor khudree*, *Chela fasciata*, *Barilius bakeri*, *B. gatensis*, *B. canarensis*, *Danio malabaricus*, *Gara mullya*, *Nemacheilus guenthari*, *N. semiarmatus*, *Lepidocephalus thermalis*, *Mystus montanus*, *M. armatus*, *M. gulio*, *Ompok malabaricus*, *O. bimaculatus*, *Hyporhamphus limbatus*, *Xenotodon cancella*, *Aplocheilichthys lineatus*, *A. dayi*, *Monopterus albus*, *Ambassis commersoni*, *Parambassis thomassi*, *Channa striata*, *C. marulius*, *Mastacembelus armatus* and *Tetraodon travancoricus*.

This paper presents the findings of the Marine Products Export Development Authority sponsored project to identify the potential ornamental fishes in the fresh water bodies in Kerala suitable for trading in international market. The paper gives a detailed account of the various fish species collected during survey, their colour plates, local name, trade name, method of fishing and survival rate in captive condition.

The survival rate of fishes collected during survey from the selected rivers was found by maintaining them in aquaria for a one-year period. Fishes with a survival of 70-80 % was categorized as high rate of survival. High rate of survival is shown by *P. fasciatus*, *B. bakeri*, *D. malabaricus*, *G. mullya* and *L. thermalis*. *P. denisonii*, *P. jerdoni*, *C. fasciata*, *N. guenthari*, *N. semiarmatus* and *T. travancoricus* showed medium rate of survival (survival rate of 50-70%). *H. limbatus*, *X. cancella*, *A. commersoni*, and *P. thomassi* showed the least survival rate (less than 50%). Fishes exhibiting high and medium rate of survival are recommended for aquarium trade as they showed survival rate for a period of 6 months to 1 year in captivity and hardy nature. The most indigenous species of Kerala in demand in the aquarium trade of national and international market are *P. denisonii*, *T. travancoricus*, *P. filamentosus* and *P. fasciatus*.

BLACK DISEASE IN LARVAL AND ADULT *Artemia urmiana* IN IRAN

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Black disease (BD) development was studied among *Artemia urmiana* larvae and adults given various feeds including algae, soybean meal, rice bran, wheat bran or yeast. The morphological distribution in size and shape and the prevalence of BD lesions was determined among infected *A. urmiana*. Detailed clinical examination of the head, thorax, genital segments, abdomen, extremities, thoracopods, antennae, claspers, telopods and tail of each affected *A. urmiana* were recorded using a compound microscope. The mean circumference of black spots in the head, uterus and extremities was calculated at two places in each *A. urmiana* examined. Moreover, BD incidence by gender was also compared. Results showed that BD was characterized by the presence of black spots in the head, uterus, extremities, thoracopods, antennae, claspers telopods and tail. The number of black spots observed per individual *A. urmiana* ranged from 1 to 10. The black spots varied from minute to many microns in diameter and varied in shape: circular, elliptical, ovate, narrowly ovate, broadly ovate, narrowly ovate, and depressed ovate and oblong. The black spots in the head and uterus were mostly irregular in shape while those in the extremities were long 159.861 ± 10.165 microns and slender 85.723 ± 4.564 microns. The prevalence of BD was higher among *A. urmiana* males (52.8%) than females (47.2%). There were significant differences in occurrence of BD in *A. urmiana* fed with the different agricultural-by products, yeasts and algae. *Artemia urmiana* fed with algae, rice bran and soybean meal showed a higher prevalence of BD than those fed with yeasts and wheat bran.

**RECENT ADVANCES IN THE DEVELOPMENT OF CAPTIVE BREEDING TECHNOLOGY
FOR THE INDIGENIOUS ORNAMENTAL FISHES OF THE WESTERN GHATS OF INDIA**

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The Western Ghats of India is well known for rich biodiversity and endemism with regard to freshwater fishes. Many of the endemic freshwater fishes have great potential as ornamental fish. But recent surveys reported depletion of stocks at alarming rate due to over exploitation and clandestine export. In this context development of captive breeding technology has great importance.

In this paper, recent developments in captive breeding technology for five species of fishes: *Puntius melanampyx*, *Puntius melanostigma*, *Puntius filamentosus*, *Chela fasciata* and *Nemacheilus semiarmatus* is discussed. Details of courtship behaviour, sexual dimorphism, fecundity, and reproductive strategies are presented in the paper. The breeding technology developed for these species can be utilized for the commercial production, which will definitely enhance production, conserve endemic and endangered germplasm and increase employment opportunities.

EFFECT OF DIFFERENT PROTEIN AND LIPID LEVELS IN BROODSTOCK DIET ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF FEMALE *Xiphophorus helleri* (POECILIIDAE)

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A study to investigate the effect of different levels of dietary protein and lipid on swordtail (*Xiphophorus helleri*), a popular ornamental fish was carried out. Eight isocaloric semi purified diets containing two protein levels (20% and 30%) with 4 lipid levels (8%, 12%, 16%, 20%) within each protein levels were fed to female broodstock for 26 weeks. Results showed that SGR (Specific Growth Rate) was significantly higher in broodstock fed with 30% dietary protein diets as compared to 20% protein. Highest SGR was obtained from diet containing 30% protein with 8% and 16% lipid respectively. Diet with 30% protein 8% lipid also showed highest feed intake. GSI (Gonadosomatic index) was highest in diet containing 30% protein 20% lipid while lowest value was obtained from the 20% protein, 8% lipid diet. Proximate analysis showed significant higher muscle protein deposition in diets containing 30% protein. Fry production was also significantly higher in 30% protein diets as compared to the 20% diets. There was no significant difference between fry production among different levels of lipid in the 30% protein diets. Diet with 20% protein 8% lipid gave the lowest relative fecundity value. Fry production was found to correlate significantly with the final weight and length of female broodstock. Fry production per feed intake was found significantly higher in diets containing 30% protein with 12%, 16% and 20% lipid. Fry production per g of protein intake also showed a similar trend as above. Meanwhile, fry production per g of lipid intake was found significantly higher in diets with 30% protein. Based on this study, we suggest 30% protein with 8% to 16% of lipid be included in to fulfill requirements for both growth and reproduction in female swordtails.

**ORIENTATION OF PIGMENT MOLECULES IN ORNAMENTAL FISHES OF
FRESHWATER AQUATIC SYSTEM AND THEIR ASSOCIATED HEALTH PROBLEMS
AND IDENTIFICATION OF SOME FEED ADDITIVE IN RESTORATION**

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Orientation of pigment molecules or carotenoids in freshwater ornamental fishes of different habitat and the effects of different environmental factors, including the presence of different xenobiotics, have revealed several health issues of the pigment molecules with reference to their restoration and biosynthesis. Ornamental fish bear carotenoid molecules that impart various colours such as red, yellow, green, pink, purple etc. Colouration occurs in their body viz. scales, skins, fins and tissues such as muscle, liver, gonads etc. Several carotenoids have been identified such as astaxanthin, β -carotene, β -apocarotenals, lutein, cryptoxanthin, canthaxanthin, tunaxanthin etc. It has been known that several factors such as enzyme (especially the deoxygenase system), hormones, gonodial maturity etc. play significant roles in the accumulation, deposition and conversion into retinol (marine and freshwater fish) or dehydroretinol (freshwater fish).

In the present communication, attempts were made to show that pigments or carotenoids molecules are affected during fasting, stress and strain conditions and in different environmental conditions (such as high turbidity, change of pH, depletion of oxygen) and in the presence xenobiotics (such as organochlorine, organophosphorus compounds and others like oils and grease). Several experiments were designed to elucidate the effects of the above-mentioned factors on the accumulation, biogenesis and metabolic role of the deoxygenase system in the entire orientation of the pigments molecules, which pose serious health problems to the ornamental fishes of freshwater aquatic system. A few feed components or precursors as feed additives, which could be added as feed additives in restoration were also identified.

ORNAMENTAL FISHERIES PROSPECT AND SCOPE: A CASE STUDY FROM ARUNACHAL PRADESH

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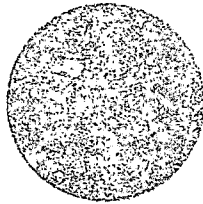
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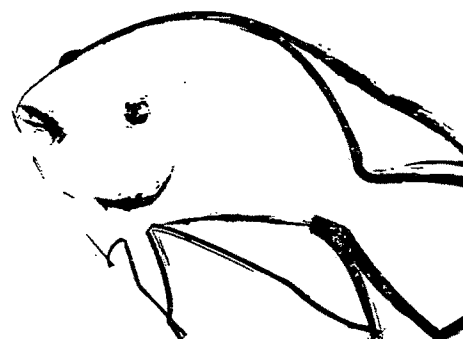
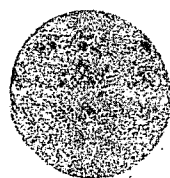
Aquaculture is growing as an industry world wide, as source of protein food, income and employment. Apart from this the colorful fishes add aesthetic value in house aquaria. The hill fisheries, particularly in the state of Arunachal Pradesh in eastern Himalayan region of India with fast flowing habitats harbors 50-60 species of fish that are of value as ornamental fish (Table-1). Of these, two representatives of genus *Amblyceps* Blyth namely *A. arunachalensis* and *A. apangi* are new to the science and hitherto never been reported from any part of the globe. Besides, there are three fish species i.e. *Labeo devdevi*, *Osteocheilus neilii* and *Colisa labiosus* which are endemic to this region and can play a pivotal role in the ornamental fish trade. Interestingly, the indigenous hill fishes have a great market demand in Europe, America and South East Asian countries. The resources have been randomly being exploited from most of the areas to such a situation that some species are under threat of extinction. The inner line entry restriction in this part of the country has conserved to some extent the fish fauna. Yet large-scale fishing for domestic consumption is going on unabatedly for want of holistic Legislation. However, the sustainability of the aquatic resources and its management depends on aqua ranching and *exsitu* rearing and breeding of the economically commercial ones.

Table 1 Habitats and major genera of wild fish fauna in Arunachal Pradesh, India

Serial No	Type of Habitats	Genera of available ornamental piscine resources in wild
1.	True hill stream forms	<i>Garra</i> , <i>Psilorhynchus</i> <i>Glyptothorax</i> , <i>Euchiloglanis</i> <i>Exostoma</i> , <i>Pseudecheneis</i> <i>Schizothorax</i> , <i>Schizopyge</i>
2.	Semi torrential forms	<i>Noemacheilus</i> , <i>Acanthocobitis</i> , <i>Botia</i> , <i>Someleptes</i> , <i>Lepidocephalus</i> , <i>Amblyceps</i> , <i>Silurus</i> , <i>Aborichthys</i> , <i>Olyra</i> , <i>Pillaia</i>
3.	Migratory forms	<i>Anguilla</i> , <i>Labeo</i> , <i>Acrossocheilus</i> , <i>Tor</i> , <i>Channa</i> , <i>Mystus</i> , <i>Badis</i> , <i>Anabas</i> , <i>Mastacembalus</i>
4.	Plain water forms	<i>Colisa</i> , <i>Chanda</i> , <i>Danio</i> , <i>Chela</i> , <i>Rasbora</i> , <i>Amblyopharyngodon</i> , <i>Barilius</i> , <i>Semiplotus</i> , <i>Puntius</i> , <i>Notopterus</i> <i>Macrognathus</i> , <i>Xenonothodon</i> , <i>Ompok</i> , <i>Mystus</i> , <i>Hara</i> , <i>Clarius</i> , <i>Heteropneustes</i> , etc.,



SS Advances in Shrimp Biotechnology II



BIOTECHNOLOGY IN SHRIMP AQUACULTURE RESEARCH: APPLICATIONS AND EXPERIENCES AT THE OCEANIC INSTITUTE

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Biotechnology is a term with a myriad of definitions. In a broad sense, it refers to the use of biological processes to produce useful organisms or products and it includes traditional biotechnologies, gene technologies and reproductive technologies. The application of biotechnology in shrimp aquaculture already has had a significant impact on the global shrimp farming industry, and there is an expectation that it will play an even greater role in the future. Researchers at the Oceanic Institute (OI, Waimanalo, HI, USA) have collaborated with "biotechnologists" in the U.S. to address fundamental challenges in the areas of shrimp stock improvement, disease diagnostics, and microbial ecology. With regard to stock improvement, there is a continuing trend among farmers to use shrimp that have been bred in captivity. A major advantage in using captive-bred shrimp is the opportunity to benefit from genetic improvement, and the use of biotechnology in genetic improvement programs can have a significant impact on genetic gain. For example, breeding programs that rely on family selection commonly use internal elastomer tags for stock identification. However, the use of these physical tags requires a large investment in labor and represents a significant bottleneck in the development of genetically superior shrimp. In collaboration with OI, researchers from Tufts University (Boston, MA, USA) have developed polymorphic microsatellites to identify families of shrimp in OI's breeding program, and this tool will enable us to increase selection intensity by evaluating thousands of shrimp per family rather than several hundred, which is the current practice. In addition to using biotechnology for selective breeding, OI is collaborating with researchers from the University of Connecticut (Storrs, CT, USA) to produce transgenic shrimp. To date, we have successfully transfected shrimp with a gene construct that code for a synthetic analog of cecropin, a broad antimicrobial peptide. Although disease-challenge tests have not yet been conducted, we hope to produce shrimp that exhibit resistance to a suite of pathogens, including those viruses which cause OIE notifiable diseases. The ability to insert foreign gene constructs into shrimp has the potential to leapfrog traditional breeding and revolutionize the global shrimp farming industry. The use of biotechnology in disease diagnostics is well established. Over the past decade, OI has collaborated with researchers from the University of Arizona (Tucson, AZ, USA) to produce and maintain specific pathogen free (SPF) shrimp using PCR techniques to screen for known viruses. This application has contributed significantly to the establishment of biosecurity protocols and procedures that are now used throughout the global shrimp farming industry. Although the use biotechnology to study the microbial ecology of natural aquatic systems is well established, it is not commonly used to study shrimp aquaculture systems. OI is collaborating with researchers from the Scripps Institution of Oceanography (La Jolla, CA, USA) to begin to understand microbial community structure and function in recirculating raceways used for intensive shrimp culture, using non-culture techniques such as denaturing gradient gel electrophoresis (DGGE) and fluorescence *in situ* hybridization (FISH). By characterizing these microbial communities, we hope to be able to manipulate carbon and nitrogen flux pathways to improve shrimp performance.

THE APPLICATION OF BIOTECHNOLOGY TO UNDERSTANDING THE EVOLUTION OF VIRULENCE IN EMERGING SHRIMP PATHOGENS

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Over the past fifteen years several highly virulent pathogens have emerged in shrimp aquaculture worldwide. Often, it is assumed that the highly virulent nature of these pathogens results from the recent association of the pathogen with the shrimp and that over time the virulence will diminish and perhaps even vanish. However, recent theories of pathogen evolution conclude that a host-pathogen association does not necessarily evolve steadily toward a more benign state but will evolve to an intermediate level that is the outcome of a balance between two opposing factors. Those factors are an assumed positive relationship between viral load and transmission and a second positive relationship between viral load and pathogenicity. The intermediate pathogenicity hypothesis results from an evolutionary balance between pressure to increase loads and thereby increase transmission but to not increase levels so high as to kill the host through those same increased loads. To determine viral loads associated with pathogenicity and with transmission in particular, a sensitive quantitative method to detect the virus is required. Although traditional assays using cultured cells have not been possible with shrimp, recent developments in biotechnology using molecular methods have led to advancements in quantitative research on viral pathogens of penaeid shrimp. For example, we used a technique termed differential display to isolate a segment of the Taura syndrome virus (TSV) genome in order to develop a molecular probe for the virus. The technique is based on the isolation of differentially expressed genes between infected and non-infected individuals. Differential display may be useful in the future for studying emerging pathogens because sequence isolation can be carried out without the need to physically isolate the pathogen and only a small amount of infected tissue is needed. Based on the TSV genomic sequence isolated using differential display, we developed a real-time RT-PCR procedure using molecular beacon technology to quantify TSV in shrimp. A study examining TSV loads during the course of infection showed a rapid increase in TSV titer in shrimp hemolymph that peaked four days after virus exposure and remained high for at least eight days, coinciding with the period of maximum mortality. Mortality declined rapidly but TSV loads remained high for 21 days with lower but persisting levels of TSV for at least 60 days. In a second study we tested the assumption that increasing the viral load in a host increases the transmission from an infected host. We identified 16 *Litopenaeus vannamei* individuals with TSV densities from 10^4 to 10^9 virions per $0.25 \mu\text{L}$ of hemolymph by real-time RT-PCR. To estimate transmission rates, those 16 shrimp were placed into a 1 m^2 bottom surface area tank with 12 uninfected susceptible *L. vannamei*. The result showed an increase in transmission rate with increasing viral density. Quantitative RT-PCR data support the assumption of a positive relationship between viral load and pathogenicity and between viral load and transmission. The results suggest that the pathogenicity of the recently emerged viral pathogens may not evolve to a completely benign state.

A PRIMARY SHRIMP CELL CULTURE SYSTEM HIGHLY PERMISSIVE FOR WHITE SPOT SYNDROME VIRUS INFECTION

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Cell growth in ovarian primary culture of the kuruma shrimp, *Marsupenaeus japonicus*, was examined under various culture conditions. Among several enzymes tested, collagenase type V exhibited the lowest toxicity for the growth and maintenance of ovarian cultured cells. Among the media and supplements tested, the best cell viability was observed in: double strength of Leibovitz-15 medium supplemented with 10% fetal-bovine serum, glucose (1 g/l), proline (0.1 g/l), TC-Yeastolate (1 g/l) and lactalbumin hydrolysate (1 g/l). The cells survived in this medium for 45 d at 25°C. Epithelial-like cells predominated in 10-d-old cultures, covering > 80% of the surface area on flask bottoms. When cell proliferation was assessed by DNA synthesis, 5-bromo-2'-deoxyuridine (BrdU)-associated cells accounted for 11.5% and 35.0% of cell populations at 2 h and 24 h, respectively. Replication of white spot syndrome virus (WSSV) was investigated in primary ovarian cultures. The virus used was a strain isolated by a limiting dilution technique in the crayfish, *Procambarus clarkii*. Sucrose-gradient purified virus was inoculated into 10-d-old ovarian primary cultures. WSSV infection produced marked cytopathy in cultured cells. Initially, virus-infected cells began to shrink 72 h p. i., followed by rounding of most cells that finally detached from the flask surface. Electron microscopy clearly demonstrated that the replication of WSSV occurred in nuclei of ovarian cells. Immunoblot analysis with antibodies against the WSSV envelope protein VP28 showed that levels of WSSV antigen in culture supernatant gradually increased between 24 and 120 h after virus inoculation. Overall results encourage us to use primary ovarian cell cultures of the kuruma shrimp for a better understanding of biochemical and molecular aspects involved in WSSV infection.

DEVELOPMENT OF GENE TRANSFER TECHNOLOGY FOR SHRIMP

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Transgenic technology is a powerful tool to study gene regulation, expression and molecular functions *in vivo* and it is possible to produce new organisms having special traits such as disease resistance. However, there are only a few reports about crustacean transgenic research. For shrimp transgenesis, it is necessary to develop a gene transfer method and methods to regulate foreign gene expression. We developed gene transfer techniques and assessed promoter activity derived from kuruma shrimp, *Penaeus japonicus* using black tiger shrimp, *Penaeus monodon* as a model crustacean species. For the regulation of exogenous gene expression, the kuruma shrimp EF-1 α gene promoter region was identified. The promoter region containing about 1,592 bp, was linked with green fluorescence protein (GFP) and chloramphenicol acetyl transferase (CAT) gene and named pJEF-GFP and pJEF-CAT, respectively. pJEF-GFP was introduced into black tiger shrimp fertilized eggs by microinjection and particle gun bombardment. By microinjection methods, the survival rate was 17.6 %, and GFP positive embryos comprised 1.0 % of the treated embryos. However, the GFP positive embryos were damaged and embryogenesis did not progress. By particle gun bombardment, the survival rate was 60.6 %, and GFP positive embryos comprised 0.42 % of the treated embryos. Ubiquitous GFP expression was observed from 8 hours post fertilization and these embryos developed and hatched normally. pJEF-CAT was introduced into black tiger shrimp fertilized eggs optimized conditions for particle gun bombardment. CAT activity was observed from 1 day to 7 days post fertilization. The highest activities were observed at 5 and 7 days post hatching. In this study, we established the activity of a ubiquitous promoter derived from kuruma shrimp and developed a gene transfer technique for black tiger shrimp.

USE OF EDIBLE ANTIBODIES FOR SHRIMP HEALTH MANAGEMENT

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Aquaculture has developed significantly, earning a considerable amount of foreign exchange but success with disease diagnosis and control lags behind industry needs. The success of any farm operation depends on its health management system to carry. To implement appropriate prophylactic and control measures, diseases affecting fish require rapid, specific and sensitive diagnostic techniques for the early diagnosis. These tests should not require sophisticated laboratory facilities and should be performed on-site. Immunodiagnostic kits are ideal for this purpose and specific antibodies are needed for their production. Egg-yolk antibodies (IgY) can help to meet this need. They also have great scope for use in passive immunization (i.e. IgY immunotherapy), one of the latest concepts in health management. Example applications of this technology include IgY ELISA kits for early diagnosis of polytrauma, sepsis and shock in clinical-surgical diagnosis and passive immunotherapy against HIV-infection (AIDS) in humans. IgY produced in chickens offers several important advantages over antibodies produced in other animals and it presents an appealing alternative to polyclonal antibodies produced by conventional methods. In addition, eggs from immunized chickens provide a continual, daily source of polyclonal antibody, and this convenient approach offers greater compatibility with animal protection regulations. Their use would reduce the use of chemicals and antibiotics in the aquatic environment. Easy availability of diagnostics would lead to faster confirmation of disease and more efficient control. Successful, passive, IgY-egg powder oral immunotherapy against bacterial and viral diarrhea in piglet, calves, rabbits and poultry, and *Salmonella*, rotavirus, coronavirus, parvovirus, adenovirus and reovirus infections in waterfowl have been reported but there is no information with respect to fisheries and aquaculture. Integration of passive IgY-immunotherapy into fisheries and aquaculture health management could help in the control of epidemics and stabilization of output. The following are the advantages of using edible antibodies:

1. Chickens are more apt than mammals to make high-avidity antibodies.
2. Humane production: no bleeding, simply collection of eggs. A single chicken can produce an enormous 3 grams of IgY per month (10-20 times the amount of a rabbit); quantities equal to 10 rabbits in 1 year.
3. Chickens produce high-titre antibody much quicker (25 days) and on less antigen than rabbits.
4. Doesn't activate complement systems or bind to mammalian Fc receptors.
5. Storing of eggs (antibodies) is very easy.
6. It is cheaper to feed and house chickens than rabbits.
7. IgY is stable and like mammalian IgG is divalent, degraded by papain to yield divalent Fab fragments and can be enzyme-labeled, biotinylated and gold-labeled by standard procedures.
8. Effective against drug-resistant bacteria.
9. Application will lead to reduced use of antibiotics.
10. Gut peptides control feed intake and gastrointestinal functions. The immune system stimulates the release of gut peptides that decrease feed intake. Fish producers often feed antibiotics, which reduce the immune stimulus.
11. Since the core body temperature of chickens is about 41°C, the IgY half-life is months at room temperature.

MONOCLONAL ANTIBODY BASED TESTS FOR DIAGNOSIS OF VIRAL AND BACTERIAL INFECTIONS IN SHRIMP AQUACULTURE

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Monoclonal antibodies against various pathogenic viruses and bacteria were successfully produced for diagnosis of infections in penaeid shrimp. These include antibodies specific for gp116, gp64, and p20 proteins of Yellow head virus (YHV), VP28 of white spot syndrome virus (WSSV), a 54 kD protein of hepatopancreatic parvovirus (HPV) and 49, 37-32, and 8 kD proteins of *Vibrio harveyi*. Various immunological techniques have been applied for diagnosis of infections by these pathogens. All of these antibodies can be used for detection of infection in shrimp tissues by means of immunohistochemistry with high accuracy and sensitivity. Dot blot is also a convenient and quick method for pathogen detection in haemolymph and tissue homogenates although in most cases sensitivity is insufficient to detect cryptic infections and high background from nonspecific binding of antibodies to high concentrations of shrimp proteins is sometimes encountered. Western blot analysis is a more specific technique with sensitivity comparable to that of dot blot. However, major drawbacks for this technique are that it is a time consuming and laborious processes. An indirect sandwich ELISA was developed successfully for YHV and WSSV due to the availability of the antigens for production of polyclonal antibodies. The sensitivity of this method is close to one step RT-PCR or PCR, and as many as several hundreds samples can be tested simultaneously at low cost. However, special equipment and skilled technicians are major requirements. Recently, a convenient test "strip test" kit was developed successfully for detection of virulent YHV infection. The sensitivity of this kit is higher than that of dot blot and no special equipment or skilled personnel are needed. As the result can be obtained within a few minutes, this type of test kit is useful for pond-side diagnosis. However, the cost per test is still high due to the small volume of production. Convenient test-strips for WSSV, gill associated virus (GAV) and *V. harveyi* are under development with targets for production before the end of 2004. Work on monoclonal antibodies against Taura syndrome virus, VP26/VP19 of WSSV, infectious hypodermal and hemopoietic necrosis virus (IHHNV) and monodon baculovirus (MBV) is ongoing together with work on other *Vibrio* species.

THE RELATIONSHIP OF A PUTATIVE POLYMERASE GENE AND TAXONOMY OF A DNA BACTERIOPHAGE OF *Vibrio harveyi*

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VHS1 (*Vibrio harveyi* siphoviridae like) phage was isolated together with *Vibrio harveyi* (VH) isolate VH1114 from a black tiger shrimp-rearing pond in the Southern part of Thailand. Classification and nomenclature of phages has traditionally depended mainly on their morphology. However, there are many examples among emergent viruses where morphological characteristics do not correspond with genomic properties. This might be due, in part, to the dynamic exchange of genetic material amongst them. As an example, an open reading frame of VHS1 phage called PH102-786 displays highly significant homology to DNA polymerase sequences at GenBank and contains conserved domains of 3'-5' exonuclease and DNA polymerase A. When used as a tool to examine the phylogenetic relationship among DNA tailed phages, it produced trees that were not compatible with those based on phage morphology. The results suggest that comparisons based on major genes such as polymerases can constitute additional criteria for evaluation of DNA phage relationships and that genome comparisons may result in more reliable taxonomy than taxonomy based mainly on phage morphology.

**DEVELOPMENT OF AFLP GENETIC MARKERS FOR FAST GROWTH AND SEX TRAITS
OF THE BLACK TIGER SHRIMP *Penaeus monodon***

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The amplified fragment length polymorphism (AFLP) technique was used to develop genetic markers that were specific for sex and fast growth traits of *Penaeus monodon*. A total of 64 primer combinations of *Eco*RI-TNN and *Mse*I-CNN were used to screen 151-day-old shrimp that were classified into 4 different groups according to their weight and sex. A total of 18 polymorphic loci were identified from 13 primer pairs. Of these, 14 markers were specific to the growth trait while another 4 were specific for sex (1 marker specific for male sex and 3 markers specific for female sex). All identified AFLP bands were re-amplified and 12 PCR product bands were subsequently cloned and sequenced in order to design primers for sequence characterized amplified region markers (SCARs). The primers were tested against pooled and individual genomic DNAs and one SCAR marker designated SF-E₅₁M₂₉ was identified with a polymorphism specific to fast growth with about 75% accuracy of phenotype prediction. The polymorphic bands of the SCAR SF-E₅₁M₂₉ were the result of an insertion/deletion of nucleotides within the amplified region. PCR products from the remaining primer pairs showed no polymorphism even after further analysis using the single strand conformational polymorphism (SSCP) technique. However, the AFLP markers that were not converted to SCARs will be useful in the construction of a genetic linkage map and the SF-E₅₁M₂₉ marker will be useful in a marker assisted selection program for fast growing *P. monodon*.

SEX-RELATED AFLP MARKERS AND GENE EXPRESSION IN ANDROGENIC GLANDS OF THE GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii*

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Sex-specific DNA markers of the giant freshwater prawn (*Macrobrachium rosenbergii*) were identified by AFLP analysis. Sixty-four primer combinations were used in a primary screen against bulk genomic DNA of the first sample set that comprised small orange claw (SOC1, $N = 10$) and blue claw (BC1, $N = 5$) males and females (F1, $N = 10$). Ninety candidate sex-specific markers from 40 informative primers were used in secondary tests with the first sample set and a second sample set (SOC2, $N = 5$; OC1, $N = 15$; BC2, $N = 10$ and F2, $N = 20$). A total of 5 male-specific and 4 female-specific AFLP markers were found. After characterization by cloning and sequencing, these were found to comprise unknown DNA segments ($E > 10^{-4}$). SCAR markers were developed from candidate sex-specific AFLP markers. Among 13 primer pairs tested, 9 generated expected amplification products from both male and female *M. rosenbergii*. Further analysis of the amplified products by SSCP analysis revealed polymorphism for all except FE₃8M₃270.2 but polymorphic patterns did not show sex-specificity. Additional primers designed from AFLP fragments of this study and from sex-related transcripts of the black tiger shrimp (*Penaeus monodon*) and the tropical abalone (*Haliotis asinina*) were applied for identification of sex-specific/differential expression markers in *M. rosenbergii*. As a result, a homologue of sarco/endoplasmic reticulum Ca²⁺ ATPase C (SERCA) was obtained using ME₃8M₃1310.1 primers ($E = 1 \times 10^{-103}$). An amplified SERCA (279 bp) revealed differential expression patterns between androgenic glands (AG) of males and oviducts of female *M. rosenbergii*. RAP-PCR analysis (150 primer combinations) was also carried out to isolate various types of expression markers in different male morphotypes and between males and females of *M. rosenbergii*. In a total, 43 male-specific and 24 female-specific expression markers and 29 differential expression markers were found. Additionally, 10, 12 and 13 morphotype-specific expression markers were identified in the AG of BC, SOC and OC males, respectively. Interestingly, SOC- (340 bp) and female-specific (315 bp) RAP-PCR markers were successfully identified from combinations of a first primer (UBC428 for SOC males and UBC122 for females) and all investigated second primers (30 primer combinations). Two markers (M122/159RAP.2 and SOC268/273RAP) derived from RAP-PCR fragments gave interesting results at the transcriptional level. For example, the expression levels of M122/159RAP.2 (a homologue of I-connection of crayfish) clearly showed differential expression patterns in AG of males and oviducts of females but no differences amongst different male morphotypes. RT-PCR (cDNA) and SSCP (genomic DNA) patterns of SOC268/273RAP implied length polymorphism through alternative splicing for this unknown gene.

GENETIC VARIATION OF *Penaeus merguensis* IN THAILAND

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Three nuclear loci were used to assess genetic variation and differentiation among populations of *P. merguensis* collected from the Gulf of Thailand and Andaman Sea. The multilocus F_{ST} revealed great and highly significant differentiation between the two seas ($F_{ST} = 0.203$, $P < 0.001$), mostly due to the polymorphism of locus PvAmy. Analysis of locus PvAmy within samples from the Gulf of Thailand revealed strong isolation by distance, indicating restricted gene flow. It also suggested the possibility that PvAmy locus could be undergoing strong environmental selection.

DIFFERENTIALLY EXPRESSED cDNA MARKERS IN OVARIES AND TESTES OF THE GIANT TIGER SHRIMP *Penaeus monodon*

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Farming of the giant tiger shrimp (*Penaeus monodon*) has relied entirely on wild broodstock, resulting in heavy exploitation of wild stocks. Moreover, breeding of pond-reared *P. monodon* has not yet produced enough larvae of the quality required by the industry. Maturation of pond-reared broodstock of *P. monodon* is extremely low. Therefore, the basic knowledge about genes expressed at different developmental stages of ovaries and testes and differentially expressed transcripts between these organs would provide a better understanding about maturation of high quality pond-reared shrimp. Subtractive cDNA libraries of ovaries and testes of *P. monodon* were constructed. A total of 216 clones (155 clones from ovaries and 61 clones from testes) were unidirectionally sequenced. Most of the expressed genes in ovaries encoded thrombospondin (TSP, 45 clones accounting for 28.7% of total expressed sequence tags, ESTs), followed by peritrophin (17 clones, 10.8%) and unknown transcripts (76 clones, 49.7%). Conversely, almost all of the ESTs in *P. monodon* testes were unknown transcripts (59 clones, 96.7%). Gender-specific expression of candidate sex-linked gene homologues and transcripts related with ovarian maturation (TSP, peritrophin, AGESTX, Usp9X and XNP-1) were examined by RT-PCR. While XNP-1 and peritrophin were expressed in both ovaries and testes, TSP, AGESTX and Usp9X homologues revealed sex-specific expression in female *P. monodon*.

**GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE GIANT TIGER SHRIMP
Penaeus monodon IN THAILAND**

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The giant tiger shrimp (*Penaeus monodon*) is one of the most economically important cultured crustaceans. Farming of *P. monodon* relies entirely on ocean-caught females for seed supply. This open-cycle and reliance on natural stocks results in heavy exploitation of wild female broodstock. Integrated knowledge on population genetics (for estimation of genetic variation), molecular genetics (for identifying genetic markers in different applications) and quantitative genetics (for domestication and selection of hatchery-propagated stocks) are required for construction of appropriate broodstock management, restocking and selective breeding programmes of *P. monodon*. Several molecular genetic approaches were used to analyze *P. monodon* genetic diversity and population differentiation in Thai waters. Mitochondrial DNA polymorphism (16S rDNA and COI-COII) revealed large genetic heterogeneity between Gulf of Thailand (east) and Andaman Sea (west) samples ($P < 0.0001$) but not within each coastal region ($P < 0.05$). This revealed a genetic population structure for *P. monodon* in Thailand. Contradictory results for *P. monodon* within the Gulf of Thailand were revealed by analysis of nuclear DNA polymorphism using microsatellites and by RAPD analyses that consistently showed a significant difference between *P. monodon* from Chumphon and Trat ($P < 0.0001$). Under a presumption of selective neutrality for genetic markers, biased female-mediated gene flow may exist between *P. monodon* in the Gulf of Thailand. In addition, molecular markers used for detection of shrimp originating from the Andaman Sea and Trat (950 bp from UBC428 and 260 bp from UBC268, respectively) and for family identification of the hatchery stocks (*CUPmo18*, *Di25*, *Di27*, *CSCUPmo1* and *CSCUPmo2*) were developed and can be applied for selective breeding of *P. monodon*. Highly polymorphic microsatellites can also be applied for individuality and parentage analysis, eliminating problems from traditional selective breeding programs where offspring of different family lines need to be cultured separately. Amplified fragment length polymorphism (AFLP) analysis is also carried out to isolate additional population and/or region-specific markers of *P. monodon*. Identified markers will be converted from AFLP to SCAR markers and used to assess population origins of natural and restocking stocks of *P. monodon*. Single-stranded conformation polymorphism (SSCP) analysis of cDNA-derived markers is being carried out for identification of single nucleotide polymorphisms (SNP) of functionally important genes in natural populations of *P. monodon* in Thai waters.

DEVELOPMENT OF MOLECULAR MARKERS FOR DIFFERENTIATION OF MORPHOLOGICALLY SIMILAR *Penaeus* SPECIES

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The giant tiger shrimp (*Penaeus monodon*) was formerly the most commercially important cultured species in Thailand. Due to problems with disease outbreaks, the white shrimp (*P. vannamei*) was introduced for commercial culture. Since post-larvae (PL) of *P. merguensis* are morphologically similar and difficult to differentiate from those of *P. vannamei*, they were sometimes sold to unsuspecting farmers as the latter species. Likewise, PL of *P. semisulcatus* is morphologically similar to PL of *P. monodon* and is sometimes sold as the latter. This is disadvantageous for farmers since the growth rate of *P. semisulcatus* is much slower than that of *P. monodon*. To assist farmers in screening PL, molecular markers for identifying penaeid species were developed. The 16S rDNA fragments of *P. monodon* ($N = 20$), *P. semisulcatus* ($N = 24$), *P. vannamei* ($N = 14$), *P. merguensis* ($N = 14$) and *P. japonicus* ($N = 7$) were amplified using the polymerase chain reaction (PCR) technique with primers 16S-F (5'-CGC CTG TTT AAC AAA AAC AT-3') and 16S-R (5'-CCG GTC TGA ACT CAG ATC ATG T-3'). The amplified fragments (approximately 560 bp in length) were subjected to restriction fragment length polymorphism (RFLP) and single-stranded conformation polymorphism (SSCP) analyses. Morphologically similar shrimp (i.e., *P. monodon* vs *P. semisulcatus* and *P. merguensis* vs *P. vannamei*) could be unambiguously differentiated. Nevertheless, results with *P. merguensis* and *P. vannamei* were not consistent when larger numbers of specimens were analysed. Therefore, a 16S rDNA fragment representative of each shrimp was cloned and sequenced and a new pair of primers giving an expected 312 bp product was designed. These were used to examine the species-origins of 86 *P. monodon* specimens, 15 *P. semisulcatus* specimens and 24 *P. merguensis* specimens originating from various locations in Thailand, 30 *P. vannamei* specimens originating from Mexico and Thailand and 16 *P. japonicus* originating from Japan. Restriction analysis of the 16S rDNA fragments from these shrimp with restriction enzymes *Alu* I, *Ssp* I and *Vsp* I revealed 3 (AAA, ABA and ABB), 1 (BBB), 2 (CBC and BBC), 1 (BAB) and 1 (BBB) mitotypes in the respective species. The amplified 16S rDNA fragment of all individuals was further genotyped by SSCP analysis. All shrimp species could be clearly differentiated. In addition, *P. semisulcatus* and *P. japonicus* that shared a BBB mitotype (PCR-RFLP) could also be differentiated unambiguously. Molecular markers developed in this study can be directly applied for quality control of traded shrimp larvae to prevent intentional supply of incorrect species.

RECENT ADVANCES IN CRUSTACEAN IMMUNITY

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Invertebrate animals do not have a true adaptive immune system and therefore have to rely on innate immune responses. During evolution, the innate immune system appeared before the adaptive immune system, and some form of innate immune reactions are likely to exist in all multicellular organisms. In contrast to adaptive immunity, innate immune recognition is mediated by germ-line encoded receptors. This means that each receptor is genetically predetermined and evolved by natural selection to have defined specificities for microbial pathogens. These microbial pattern receptors will trigger activation of innate immune responses such as the prophenoloxidase activating (proPO) system present in the blood cells of invertebrates, once they have identified and bound to pathogen-associated molecules such as lipopolysaccharides and peptidoglycans from bacteria or beta-1,3-glucans from fungi. Activation of the proPO-system is both very rapid and efficient and a concentration as low as 10 pg per litre of microbial polysaccharides is enough to trigger activation. Following activation of this enzyme cascade, several biological functions are induced that aid in the elimination of foreign intruders. Antibacterial peptides are ubiquitous and are probably of great importance for the well-being of many invertebrates. These peptides and their induction have been studied in great detail, primarily in insects and in particular in *Drosophila*, but to a lesser extent in crustaceans. The blood cells are vital for the innate immune responses since they contain the proPO-system, express antibacterial peptides, are involved in the clotting system and are the cells that remove foreign particles and pathogens by phagocytosis or encapsulation reactions. Recently we have studied how blood cells are synthesized in crayfish and we have isolated and characterised some of the genes involved. We can also block haematopoiesis by RNAi both *in vivo* and *in vitro* using a stem cell culture. Thus, we can use this stem cell culture system to study in detail how these cells mature to blood cells and how crustaceans interact with viral pathogens.

SPECIFICITY IN THE SHRIMP IMMUNE RESPONSE REVEALED BY DIFFERENTIAL DISPLAY

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In penaeid shrimp, several proteins have been described as being involved in defense against pathogens. Although shrimp hemocytes are a very dynamic tissue that makes possible recognition, phagocytosis, degranulation and nodule formation during an immune response, little is still known about their gene expression. In this work, we found that hemocytes from *Litopenaeus vannamei* were not only capable of recognizing foreign material but also distinguishing between different types. By means of differential display, it was shown that hemocyte gene expression differed upon challenge with abiotic particles or potentially-pathogenic bacteria (*Vibrio alginolyticus*) and that some of the newly expressed genes were exclusive for a specific challenge.

MOLECULAR BIODEFENSE MECHANISM IN KURUMA SHRIMP AND BLACK TIGER SHRIMP

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A basic knowledge of biodefence genes and their mechanisms of action in shrimp is still limited, despite of their extreme importance in combating microbial infections of cultured shrimp. Shrimp have an innate immune system that includes cellular and humoral defense mechanisms. An expressed sequence tag (EST) analysis was conducted for the discovery of biodefence-related genes of kuruma shrimp haemocytes. We cloned and fully sequenced several biodefence-related and immune-related cDNAs including α 2-macroglobulin (α 2M), β -1, 3 glucan binding protein (BGBP), crustin, kazal inhibitor, kunitz-type inhibitor, lysozyme, prophenoloxidase (proPO), serine protease homologue, transglutaminase (TGase), and a translationally-controlled tumor protein homologue. The results of real-time PCR analysis indicated that the expression levels of several biodefence genes dramatically increased after oral administration of the immune-stimulant peptidoglycan (PG) derived from *Biofidobacterium thermophilum*. We constructed a cDNA microarray comprised of 718 and 308 different cDNAs from black tiger shrimp, *Penaeus monodon* and kuruma shrimp, *Marsupenaeus japonicus*, respectively. Changes in the gene expression profile in kuruma shrimp or black tiger shrimp hemocytes after oral administration of PG, oxytetracycline (OTC) or oxolinic acid (OA) were analyzed using this cDNA microarray. Gene expression levels of many genes changed at 1, 3 and 7 days after administration of PG, OTC or OA. Interestingly, expression of several defense-related genes was up-regulated by the immune stimulant PG. In contrast, expression of several defence-related genes was down-regulated upon antibiotic administration. We will discuss the details of the results of microarray study for gene expression profiling of biodefence-related genes in kuruma shrimp and black tiger shrimp in this seminar. All our studies indicate that microarray analysis is a powerful method to characterize varying gene expression for a better understanding of the shrimp biodefence network system.

EXPRESSED SEQUENCE TAG ANALYSIS FOR IDENTIFICATION OF HEAT RELATED GENES IN THE GIANT TIGER SHRIMP *Penaeus monodon*

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To cope with deleterious effects of stress, living organisms have developed molecular responses for repair and protection against damage by the synthesis a number of stress proteins. These proteins assist the folding of nascent polypeptide chains, act as molecular chaperones and mediate repair and degradation of altered or denatured proteins. Because of the obvious importance of stress responses, attention has recently focused on the role of heat stress proteins in the control of disease and in improving resistance against virulent pathogens. The connection between stress responses and disease resistance has been reported in various domesticated animals, and knowledge about heat stress protein functions in vertebrates is much further advanced than in invertebrates. To identify heat response genes and their involvement in disease resistance, expressed sequence tag (EST) analysis of a complementary DNA library from haemocytes of heat stressed shrimp (*P. monodon*) was performed. From this library, 1090 cDNA clones were single-pass sequenced from the 5' end and a BLAST analysis was performed against the GenBank database. Sequence comparison revealed about 63% significant match to known genes, many of which were involved in basic metabolism and cellular organization. Some of the matched ESTs showed expected tissue specificity. A total of 134 (12.3%) expressed sequence tags (ESTs) corresponded to heat related genes. A number of these cDNA clones and EST sequences were subjected to full-length sequence and functional analyses. The data generated will be a valuable resource in further studies on the biology of *P. monodon* and on the identification of genes that can serve as potential targets for disease control.

STRESS RESPONSES IN GIANT TIGER SHRIMP *Penaeus monodon*

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The connection between stress responses and disease resistance has been reported for various domesticated animals. Acute stressors can have both immunostimulating and immunosuppressive effects depending on species and physiological status. Heat shock proteins (HSP), one of the stress-protein families, have been known to play an essential role in cell protection. HSP involved with both innate and adaptive immunity have been reported from various organisms. Induction of HSP has also been observed after infection of cells with a variety of bacteria or viruses. The aim of this study was to investigate HSP and a number of thermal-induced genes in immunotolerance of *P. monodon*. The experiment was conducted by applying sub-lethal cold and heat shock conditions (15, 30, 33 and 35 °C for 6 h) to shrimp pre-acclimated at ambient temperature (28°C). Then the shrimp were brought back and maintained at ambient temperature. Tissues and haemocytes were collected from each shrimp at 0, 3, 6, 12, 24 and 72 h after thermal induction. Profiles of protein extracts from each tissue were analysed using SDS-PAGE, followed by Western blot analysis using antibodies against human HSP60, bovine HSP70 and water mold HSP90. No differences in bands were seen on Coomassie stained gels, but Western blot analysis revealed higher levels of HSP70 from heat-treated shrimp, indicating inducibility of HSP70 in *P. monodon*. However, no binding was seen with HSP60 and HSP90 antibodies in Western blots, indicating their absence or insufficient cross-reactivity with shrimp tissue extracts. When heat-induced shrimp were challenged with *Vibrio harveyi*, their survival was significantly higher than that of uninduced control shrimp indicating that heat induction is related to immunotolerance in *P. monodon*. Using RAP-PCR, a number of DNA bands were differentially displayed between heat-induced and control shrimp. Each band was cloned, sequenced, and subjected to further characterization. A number of genes coding for HSP60, HSP70, and HSP90 in *P. monodon* were partially cloned and sequenced. Ongoing work includes full-length sequence determination and semi-quantitative analysis of HSP60, HSP70 and HSP90 expression in heat-induced shrimp. The results will provide some knowledge on the role of HSP in the immune system of *P. monodon*.

IMMUNE RELATED WAP DOMAIN-CONTAINING PROTEINS FROM *Litopenaeus vannamei* HEMOCYTES

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The WAP domain is found in proteins related to the shrimp immune system and mainly to those with antibacterial or proteinase inhibitory activity. A cDNA clone coding for a single WAP domain (SWD) protein was isolated from a hemocyte cDNA library of *L. vannamei*. The full-length cDNA sequence was 0.4 Kb long and encoded a 93-amino acid protein. SWD protein mRNA levels were modified after injection of *Vibrio alginolyticus*, indicating the probable role of SWD in the immune response. Although the amino acid sequence was similar to WAP domain-containing proteinase-inhibitors, shrimp SWD protein did not have any other functional domain. Furthermore, single WAP motif (SWAM) proteins from mice have been reported to have antibacterial activity. Phylogenic analysis showed that SWD was more related to other WAP proteins than to mouse SWAM. On the other hand, penaeid crustins in *Litopenaeus vannamei* and *L. setiferus* have been described by structural similarity to belonging to an antibacterial WAP domain-containing protein family. Six crustin-coding clones were isolated from a cDNA library from *L. vannamei* hemocytes, sequenced and compared. Two different isoforms (named I and P) were found, based on two nucleotide differences that produce one change in amino acid sequence (Ile/Pro). The mRNA steady state level of crustin I, but not crustin P was down regulated by *Vibrio alginolyticus* inoculation. Thus, a difference of one amino acid substitution among penaeid crustins seems to be associated with differential expression upon bacterial challenge.

GENE EXPRESSION IN HEMATOPOIETIC TISSUE OF BLACK TIGER SHRIMP *Penaeus monodon* BY THE EST APPROACH

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Circulating haemocytes of invertebrates play an important role in immunity and their number can vary and decrease dramatically during infection. Production of haemocytes in crustaceans occurs in organs comprised of hematopoietic tissue (HT). In shrimp, HT proliferates vigorously as evidenced histologically and is distributed in the head area. There are very few reports on HT in shrimp. In this study, we used the EST approach to gain an improved understanding of the function of HT in shrimp hoping that it would benefit the development of rational strategies to improve health management in shrimp aquaculture. Broodstock used for this study were checked to be free of white spot syndrome virus (WSSV) by nested PCR and yellow head virus (YHV) types 1 and 2 by nested RT-PCR. A cDNA library was constructed at the efficiency of 4.7×10^5 pfu/ml. A total of 931 clones were sequenced. Of these total ESTs, 469 (53%) did not match sequences in GenBank. The remaining 439 ESTs (47%) showed significant homology to deposited sequences. The matched ESTs represented 147 different deduced protein types in 11 categories according to their major functions. Of these, 24% were of mitochondrial origin, indicating contamination from mitochondria during the process of library construction. Constructing a normalized cDNA library is one possible way to improve the quality of the library and obtain a higher number of genes to study. Several of the immune genes found in HT have been reported from circulating shrimp hemocytes. These include proteinase inhibitors, transglutaminase enzyme and clotting protein. The results suggest that these immune proteins are produced and stored in young hemocytes in HT before the cells are released into circulation. Several genes involved in developmental processes in other animals (e.g. Mago-nashi and NAS proteins) were found for the first time in crustaceans. Apoptosis inhibitor involved in apoptosis (a key process in the shrimp response to viral infection) was also found. The occurrence of a high number of unmatched ESTs (53%) probably resulted from the fact that a comparatively small number of genes have been described from invertebrate tissues. Further study is aimed at elucidating shrimp genes involved in hematopoiesis and its regulation.

SERINE PROTEINASE AND KAZAL INHIBITOR FROM *Litopenaeus vannamei* HEMOCYTES

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Shrimp are able to successfully destroy pathogens using their defense system. Although specificity of the immune system seems to be limited, it can distinguish self and non-self material and establish a response through cellular and humoral mechanisms. Two examples of multimeric response systems are the pro-phenoloxidase (proPO) activation system and the coagulation system. In both systems, proteinases play a decisive role in the activation. However, their activities can be regulated to control tissue damage by means of specific serine proteinases that act as inhibitors. A serine proteinase clone and a Kazal-inhibitor clone were isolated from a hemocyte cDNA library of *Litopenaeus vannamei* and their expression profiles were examined upon *Vibrio* challenge. Structural characterization showed that the serine proteinase had, besides a catalytic domain, a clip domain that is characteristic of proteinases from arthropod hemocytes. Although, the *L. vannamei* clip domain had only 4 of the 6 typical cysteines, a similar structure was also found in *Penaeus monodon* indicating its probable common distribution in penaeid shrimp. The Kazal inhibitor contained four typical domains, two of them with probable inhibitory activity. Its mRNA expression profile was modified by *Vibrio* challenge. Structural comparison with Kazal proteins from crayfish revealed significant differences but a distant common ancestor.

MOLECULAR CHARACTERIZATION OF SHRIMP WHITE SPOT SYNDROME VIRUS (WSSV) BY GENOMIC AND PROTEOMIC APPROACHES

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White spot syndrome virus (WSSV), one of the most devastating viral pathogens of cultured shrimp worldwide, is an enveloped, ellipsoid, large double-stranded DNA virus. Recently, the International Committee on Taxonomy of Viruses approved a proposal to erect WSSV as the type species of the genus *Whispovirus*, family *Nimaviridae*. The complete genome sequence of white spot syndrome virus (WSSV) for three isolates has been determined. For the Taiwan isolate, the size of the genome is 307,287 bp, and sequence analysis identified a total of 532 putative ORFs that comprise 60 codons or more. Microarray analyses have shown that at least 89.5% (476/532) of the WSSV ORFs are expressed in WSSV-infected gill tissue and this provided a foundation for studying virus gene expression and regulation. A time course analysis using the microarray identified 4 genes (*wssv194*, *wssv313*, *wssv369* and *wssv425*) associated with the carrier stage of WSSV infection (also known as latent or persistent infection). However, since WSSV targets many other cell types in addition to gill tissue and since gene expression is often different in different tissues, we are gaining a more complete understanding of the replication strategy of WSSV using other cell types as microarray analysis targets. Although the virion protein profile of a virus is important, the large size and complexity of the WSSV virion makes such a profile difficult to obtain. The uniqueness of proteins encoded by WSSV ORFs has meant that until recently, only ~5% of the 532 WSSV ORFs with >60 amino acids have been identified as genes with known function. In a study designed to identify as many structural proteins as possible, by emphasizing completeness at the expense of absolute purity, we were able to use gradient SDS-PAGE separation of intact WSSV virions to produce the most comprehensive profile published to date. Furthermore, intactness of the WSSV virions ensured that this profile was both consistent (in terms of the molar ratios of the bands) and repeatable. After separation and in-gel trypsin digestion, LC-nanoESI-MS/MS techniques identified 33 WSSV structural proteins from 34 bands. In addition to nineteen WSSV structural proteins previously reported (six of these not detected in our study), twenty additional proteins were identified, bringing the total number of reported WSSV structural proteins to 39. One of these, structural protein VP664, is the largest viral structural protein ever found. This proteomic analysis has not only placed the structural proteins of WSSV on a genetic basis and opened the way for further studies, but by combining this technique with immunoelectron microscopy, we also demonstrated that, contrary to earlier reports, VP664 is the only major nucleocapsid protein. Meanwhile, to better elucidate the interactions between the virus and its host[s], we conducted a comparative analysis of differentially expressed genes in non-infected and WSSV-infected *Penaeus monodon* postlarvae. Two cDNA libraries, namely PmTwN (non-infected) and PmTwI (WSSV-infected), were constructed. No normalization was applied to these two libraries. A total of 7,200 and 8,064 clones were randomly selected from PmTwN and PmTwI libraries, respectively, and sequenced to generate an EST database. When ESTs from both libraries were compared with the WSSV whole genome sequence, 439 PmTwI ESTs were identified as WSSV genes, but there were no PmTwN ESTs in homolog to the WSSV genome sequence. No homologs to other shrimp viruses (YHV, IHNV, TSV) were identified in either library. These results are consistent with our initial PCR screening results, and confirmed that the shrimp used were free of WSSV as well as YHV, IHNV and TSV. Out of several hundred ESTs that were found to be homologs to known genes, 55 were identified as candidates for being transcriptionally up-regulated after WSSV infection, while 76 unique genes were identified as potentially down-regulated transcripts. These candidate genes were responsible for housekeeping, defense, growth and other biological functions. In addition to these genes, many other known and novel unique sequences were also differentially expressed.

WHITE SPOT SYNDROME VIRUS AND CRAYFISH: REPLICATION IN A CELL CULTURE AND HOST INTERACTION

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Crustaceans lack immune memory and therefore have to rely on innate immune reactions. One important innate immune defence process is the so-called proPO-system that generates several factors to aid in the elimination of foreign particles or parasites. It is a non-self recognition system that is activated by microbial products. This system has been studied in greatest detail in freshwater crayfish and most of the proPO-components have been purified and cloned. Recently, we have also been able to clone several proPO-components from shrimp species, and particularly *Peneaus monodon*. Cellular immune reactions and hence blood cells are important in defence and we have therefore isolated stem cells from haematopoietic tissue and made proliferating cell cultures from them. This allows us to study how the blood cells differentiate from stem cells to mature blood cells. We can also use these cell cultures to replicate white spot syndrome virus (WSSV) and study how the virus interacts with crustacean cells. The interaction between WSSV and crayfish has been studied both *in vivo* and *in vitro* using the proliferating stem cell cultures. By subtractive hybridisation, several genes have been isolated that are upregulated in response to the virus. A few of these have been studied in more detail to investigate their role during virus replication in cell cultures.

GENOMIC AND PROTEOMIC ANALYSIS OF 39 STRUCTURAL PROTEINS OF SHRIMP WHITE SPOT SYNDROME VIRUS

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WSSV virions were purified from the hemolymph of experimentally infected crayfish *Procambarus clarkii* and their proteins were separated by 8–18% gradient SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) to give a protein profile. The visible bands were then excised from the gel, and following trypsin digestion of the reduced and alkylated WSSV proteins in the bands, the peptide sequence of each fragment was determined by liquid chromatography (LC) nano-electrospray ionization (ESI) tandem mass spectrometry (LC-nanoESI-MS/MS) using a quadrupole/time-of-flight (Q-TOF) mass spectrometer. Comparison of the resulting peptide sequence data against the NCBI nr database identified 33 WSSV structural genes, twenty of which are reported here for the first time. Since there were six other known WSSV structural proteins that could not be identified from the SDS-PAGE bands, there must therefore be a total of at least 39 (33 + 6) WSSV structural protein genes. Only 61.5% of the WSSV structural genes have a polyadenylation signal, and preliminary analysis with 3' RACE suggested that some structural protein genes produced mRNA without a poly (A) tail. Micro array analysis showed that gene expression started at 2, 6, 8, 12, 18, 24 and 36 hpi for 7, 1, 4, 12, 9, 5 and 1 of the genes, respectively. Based on similarities in their time course expression patterns, a clustering algorithm was used to group the WSSV structural genes into four clusters. Genes that putatively had common/similar roles in the viral infection cycle tended to appear in the same cluster.

VP664, THE LARGEST VIRUS STRUCTURAL PROTEIN EVER FOUND, IS THE MAJOR NUCLEOCAPSID PROTEIN OF WSSV

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White spot syndrome has caused serious damage to worldwide shrimp culture. The causative agent, white spot syndrome virus (WSSV), contains a rod-shaped enveloped nucleocapsid enclosing a large circular double-stranded DNA genome of about 300 kbp. Analysis of the complete WSSV genome sequence suggested the presence of a giant ORF of 18,234 nucleotides that encoded a long polypeptide of 6077 amino acids. SDS-PAGE of purified WSSV virions and an LC-MS/MS database search revealed that this giant polypeptide, designated VP664, was a WSSV structural protein. Sequencing analysis of RT-PCR products confirmed the existence of the long, corresponding 18 kb transcript and also revealed that the giant ORF was intron-less. Time course analysis showed that this transcript was actively transcribed at the late stage of viral development, suggesting that it was involved primarily in the virion assembly and morphogenesis. Several polyclonal antibodies against this giant protein were prepared and one was used for IEM analysis to localize it in the virion. Immunoelectron microscopy with gold-labeled secondary antibody revealed gold particles randomly distributed at the periphery of the nucleocapsid, suggesting that the giant ORF encoded a major viral nucleocapsid protein with a periodicity that matched the characteristic stacked ring subunits that appear as striations by negative staining transmission electron microscopy. From this and other evidence, we argue that this giant ORF encodes the major WSSV nucleocapsid protein.

USE OF THE YEAST-2-HYBRID SYSTEM TO ISOLATE OF SHRIMP PROTEINS THAT INTERACT WITH TAURA SYNDROME VIRAL CAPSID PROTEIN

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Taura syndrome virus (TSV) is known to infect a number of penaeid shrimp species. However, it has caused serious economic losses only for the *Penaeus vannamei* farming industry. Thus, it would be interesting to know how each shrimp species responds to TSV and other viruses at the molecular level. The yeast-two-hybrid assay system can be used to study protein-protein interactions. The aim of this study was to examine the possibility of using it to study the shrimp interaction with viral proteins. The TSV capsid protein was used as a to test this system. Host-viral interaction in yeast cells was investigated by constructing a system containing VP1 that coded for TSV capsid as the bait and a *P. monodon* cDNA library as the prey. Two immune-related genes were identified in the initial screening and their protein-protein interaction with VP1 is being confirmed using a GST-pull down technique. Isolation of the corresponding genes from *P. vannamei* and testing of their possible interaction with VP1 is also under investigation. This is the first report of the successful use of the yeast-two-hybrid system to study protein-protein interactions in shrimp.

HIGHLY EXPRESSED GENES IN LYMPHOID ORGAN OF HEALTHY AND *Vibrio harveyi* CHALLENGED SHRIMP *Penaeus monodon*

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The lymphoid organ (LO) in penaeid shrimp is responsible for the removal of foreign material from the haemolymph before it goes from the arterial system into the open circulatory system. In this study, expressed sequence tag (EST) analysis was used to investigate gene expression in this tissue. Two standard cDNA libraries were constructed, one from LO of healthy shrimp and one from LO of *V. harveyi*-challenged shrimp (*Penaeus monodon*). To identify rare transcripts, a normalized lymphoid cDNA library was also established. A total of 448, 645 and 595 EST clones were generated from the respective libraries. Comparison of EST clones with sequences at GenBank revealed approximately 40-50% matched EST. Of these, 23 different immune-related genes were found. Interestingly, cathepsin clones were prevalent in the LO libraries and peritrophin clones were present exclusively in the challenged library where they were the most prevalent. Cathepsin clones were abundant in LO of healthy shrimp (6.3%) and also in the *V. harveyi*-challenged library (1.7%). Based on EST clustering, there were 8 and 6 different groups of the cathepsins and peritrophins, respectively. The LO peritrophins were similar to ovarian peritrophins. The level of cathepsin and peritrophin expression was studied by RT-PCR in eyestalks, gills, the heart, the hepatopancreas, hemocytes, LO and intestine of healthy shrimp. Highest expression occurred in the LO and intestine, whereas peritrophin expression was highest in the intestine. A low level of expression of both genes was found in the remaining tissues. Peritrophin expression increased in response to *V. harveyi* challenge, whereas cathepsin expression decreased. The differential expression of cathepsins and peritrophins during bacterial challenge suggests that they play some role in the response to bacterial invasion.

CLONING AND EXPRESSION OF YHV GLYCOPROTEIN GENE GP116 USING THE BACULOVIRUS EXPRESSION APPROACH

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Yellow head disease was first reported in farmed *P. monodon* in Thailand in early 1990. When it first emerged, this disease was the most virulent viral infection ever reported for the black tiger shrimp in that farmers could experience total crop loss within 3 to 5 days of the first appearance of visible signs of infection. The causative agent, Yellow-head virus (YHV), is (+) sense, single-stranded RNA virus that has been classified in a new family *Roniviridae* and genus *Okavirus* within the order *Nidovirales*. Virions are rod-shaped, enveloped particles (approx. 70 x 180 nm) with prominent surface spikes and a helical nucleocapsid. The complete YHV genome of 26,662 nt (excluding polyA tail) comprises 5 long open reading frames (ORF1a, ORF1b, ORF2, ORF3, and ORF4). ORF1a and ORF1b encode a non-structural replicase polyprotein. ORF2 represents nucleocapsid protein and ORF3 has been reported to encode two structural glycoproteins, gp116 and gp64. Glycoprotein gp116 contains two transmembrane domains at the C-terminus whereas glycoprotein gp64 contains one transmembrane domain. Here, we describe a cloning and expression strategy for the larger glycoprotein gp116 without its C-terminal transmembrane domains (i.e., truncated gp116) by using the Bac-to-Bac® (Invitrogen) baculovirus expression system. PCR products representing the truncated gp116 glycoprotein sequence and plasmid pEXPORTHIS (kindly provided by Dr Rosanne Casu, CSIRO Plant Industry, Australia) were digested with *Sall* and *NotI*, gel purified and ligated to insert the gp116 sequence downstream of the polyhedrin promoter. The recombinant transfer vector was transformed into *E. coli* DH5 α competent cells. A construct with the correct sequence was subsequently transformed into DH10Bac™ *E. coli* (Invitrogen) for transposition into a bacmid. White colonies were selected and recombinant bacmids were extracted and used to transfect *Spodoptera frugiperda* (Sf9) insect cells. At 4 days post-infection, supernatant was collected, centrifuged to remove cell debris, and analyzed by 10% SDS-PAGE in parallel with negative (no infection) controls. Gels were stained with Coomassie blue or transferred to nitrocellulose membranes. Immunoblot detection using YHV polyclonal rabbit antiserum revealed a prominent band at a size relatively smaller than 116 kDa, corresponding to the required truncated form of the glycoprotein.

DEVELOPMENT OF LOW-COST-DETECTION METHODS FOR ON-FARM SCREENING OF GILL-ASSOCIATED VIRUS (GAV) LOAD

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Gill associated virus (GAV) is a single-stranded, positive sense RNA virus that has been associated with massive mortalities in the Australian prawn farming industry. GAV is closely related to yellow-head virus (YHV) that was originally isolated in Thailand. The two viruses share 85.1% nucleotide identity while isolates of GAV share 98.5%, indicating that YHV and GAV represent distinct genetic lineages and geographical topotypes. The current method used for detecting these viruses is reverse transcriptase-polymerase chain reaction (RT-PCR) assay with specific or multiplex primers. Although the method is both specific and sensitive to the target genome, there are practical limitations to wide spread application. These limitations include the need for specialised equipment (PCR lab setup cost approx. \$15,000 AUS), well-trained personnel and high cost of processing individual samples. Consequently, most farmers decide not to monitor on-farm disease status with respect to GAV/YHV. The objective of this project was to develop low-cost detection methods for GAV (and by inference for YHV) for small-scale prawn farmers in Australia and developing countries. We have developed two alternative, non-molecular detection methods for the detection of GAV and YHV. A semi-quantitative, low cost test based on haemagglutination of chicken erythrocytes was developed to indicate the presence and viral load within *Penaeus monodon*. The study tested haemolymph, gills, the lymphoid organ, the heart, subcutaneous tissue, eye stalks, pleopods, uropods and the central nerve cord for agglutination activity in 100 prawns. Haemolymph and gill tissue gave the highest end-point titres of 1:1370 and 1:361 respectively. The sensitivity of the test was demonstrated by testing two different populations of *P. monodon* that showed a highly significant difference ($P < 0.001$) in haemagglutination activity, indicating a difference in viral load. By testing three other penaeid prawn species (*Penaeus esculentus*, *Penaeus merguensis* and *Penaeus longistylis*) and the crayfish *Cherax quadricarinatus* ($n = 20$), it was demonstrated that natural agglutinins did not cause high agglutination in the populations of *P. monodon* tested. Monoclonal antibodies (MAbs) and polyclonal antibodies (PAbs) have been developed towards GAV for the use in an enzyme linked immunosorbent assay (ELISA). The specificity of the MAbs was demonstrated by immunohistochemistry showing a positive immunoperoxidase reaction in the lymphoid organ of *P. monodon* that were also RT-PCR positive for GAV while showing a negative immunoperoxidase reaction in the lymphoid organ of *Penaeus merguensis* that were RT-PCR negative for GAV. Specificity was also demonstrated by applying immunoprecipitation to purified virus from *P. monodon* using the developed MAbs. Immunoprecipitation was positive for GAV and negative for Mourilyan virus (MoV), for infectious hypodermal and haematopoietic necrosis virus (IHHNV) and for crustacean DNA that was used with specific RT-PCR or PCR tests. We then used the optimised ELISA to test for GAV with gill samples of *P. monodon* ($n = 100$) and compared individual results to RT-PCR for sensitivity and cost analysis.

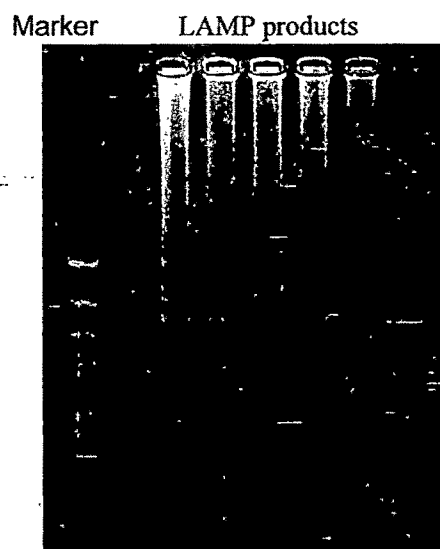
DETECTION OF WHITE SPOT SYNDROME VIRUS AND YELLOW HEAD VIRUS IN SHRIMP BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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Loop-mediated isothermal amplification (LAMP) is a novel, sensitive and rapid technique that can be applied for disease diagnosis in aquaculture. Using LAMP and RT-LAMP methods, highly specific and sensitive diagnosis systems for white spot syndrome virus (WSSV) and yellow head virus (YHV) detection were designed. A set of four primers, two outer and two inner primers, were designed from WSSV genomic DNA. Reaction time and temperatures were optimized for 60 min at 65°C, respectively. The detection limit using the LAMP method was up to 1 fg, when compared to 10 fg by nested polymerase chain reaction (PCR). Thus, the standardized LAMP procedure was used to detect the presence of WSSV in the heart, stomach and lymphoid organ from infected shrimp. A set of four primers for the detection of YHV were selected from the YHV genomic sequence. Optimum temperature and reaction time for the RT-LAMP were 65°C and 60 min. The virus RNA was extracted from heavily infected shrimp gills with either and RNA extraction kit or 0.5 M NaOH and diluted 100-fold for use as an RT-LAMP template. This study developed a diagnostic procedure that is rapid, simple and highly sensitive for WSSV and YHV detection in shrimp. The work was supported in part by a grant from "Program for Strategic Regional Science & Technology Advancement", Miyazaki Prefecture, Japan.

Figure 1 Typical agarose gel electrophoresis pattern of LAMP reaction products from WSSV. RT-LAMP products show a similar pattern.



PERSISTENT VIRAL INFECTION (VIRAL ACCOMMODATION) REDUCES THE SEVERITY OF DISEASE IN CRUSTACEANS AND OTHER ARTHROPODS

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Comparatively little published information is available on the mechanistic response of arthropods to viral pathogens. Much of the literature has been focused on the use of viruses for biological control of insect pests and disease vectors or on the use of baculoviruses as a means of heterologous protein expression in insect cell lines. The situation has changed dramatically with the rapid global increase in cultivation of penaeid shrimp and the massive farm losses that have occurred due to viral pathogens. Urgency to solve these problems has led to a closer examination of the shrimp response to viral pathogens in the hope of finding new methods of disease control. Field observations and results of laboratory experiments in the past decade indicate that shrimp are capable of a specific, adaptive response to viral pathogens that cannot be explained by current knowledge and understanding of their cellular and humoral defenses. Hallmarks of this response are specific tolerance to single and multiple viral infections without gross or histological signs of disease. These phenomena are common to crustaceans and insects and the concept of viral accommodation was introduced in 1998 as a simple testable hypothesis to explain them. Key elements of the hypothesis were an unknown mechanism for specific memory of pathogens and a role for this memory in dampening viral-triggered apoptosis. Recent field and research results have supported predictions of the viral accommodation hypothesis and suggest that memory is provided by the viral pathogens themselves in persistent infections that reduce the severity of disease. The well-known phenomenon of defective interfering viral particles probably plays an important role in this process, but it cannot explain cross protection that has recently been described for heterologous viral infections. Homologous and heterologous reduction in disease severity as a result of persistent viral infections may be a key process that has evolved from host viral interaction in the arthropod line.

THE INTERACTION OF AN OSMOLYTE, BETAINE AND SHOCK PROTEINS IN VIRUS INFECTED *Penaeus monodon*

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It has been hypothesized that shock precipitates mortalities in virus infected *Penaeus monodon* via uncontrolled apoptosis. In the case of osmotic shock, osmolytes including betaine should be able to protect against these mortalities. To test the protective effect of betaine against hypo-osmotic shock, *Penaeus monodon* were divided into 2 groups, one fed normal commercial diet and one group fed the same diet with a partially water-stabilised betaine based supplement at 1%. Both treatments were hypo-osmotically challenged and the levels of their shock proteins (SP) were measured as an indicator of stress. The salinity of the water was decreased from 35 ppt to 15 ppt over 45 min by the addition of distilled water, and maintained at this salinity for 2 h. It was then increased to 35 ppt over 30 min by the addition of seawater. The prawns were held at this salinity for 6 h before the pleopods were removed for sampling. The stress of transport from the farm to the experimental facilities enhanced mortalities. Betaine had a significant positive effect on survival in both a population of prawns in glass tanks ($\chi^2 = 10.67$; $df = 1$; $P < 0.005$), and a population housed in 1 tonne tanks ($\chi^2 = 3.84$; $df = 1$; $P < 0.05$). The survival attributable (inverse AR1) to being fed betaine was 13.5%. The levels of SP in the 2 groups were tested by the conservative, nonparametric Mann-Whitney U test. There was a significantly higher level of SP in the betaine-fed prawns than the controls ($U = 565.5$, $N = 80$, $P = 0.024$). However, a group of very low responders to osmotic shock failed to produce shock proteins. Excluding this group of non-responders from the analysis removed 3 prawns from the betaine group and 13 from the control group. Analysis with a t-test produced no significant differences ($t = 0.383$; $df = 62$; $P = 0.703$) between the betaine-fed versus the control groups. This unequivocally showed the control group had its mean SP levels lowered due to the non-responders to osmotic shock. The relative risk for failing to respond to osmotic shock when fed betaine was 0.23. This was less than 1, indicating that feeding betaine was beneficial or sparing as a risk factor. The odds ratio of failing to respond to osmotic shock when not fed betaine was 5.94 to 1 compared with being fed betaine. The lessening of risk of not responding to osmotic shock attributed to being fed betaine in absolute numbers was 25 animals out of every 100. Betaine and other osmolytes lessen the stress from environmental challenges because they protect protein from denaturation. Therefore, SP induction to refold and protect damaged proteins is lessened when osmolytes are abundant. When an animal has low betaine or other osmolytes, the production of SP becomes a critical response for protection. However, viruses slow down the production of SP because of competing mRNAs. The higher the viral load, the greater the inhibition. Therefore, prawns infected with viruses become sensitive to environmental shock and without adequate levels of protective osmolytes, they would be expected to succumb to uncontrolled apoptosis when shocked.

ISOLATION AND CHARACTERIZATION OF GENES INVOLVED IN APOPTOSIS FROM WSSV-INFECTED SHRIMP *Penaeus monodon*

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Apoptosis or programmed cell death (PCD) is considered to be a universal, essential mechanism for the elimination of unwanted or potentially harmful cells. Previous studies revealed that moribund shrimp infected with white spot syndrome virus (WSSV) and yellow head virus (YHV) have high numbers of apoptotic cells in vital tissues such as hemocytes, gills, the heart and lymphoid organ. Thus, PDC may be involved in causing shrimp death. The aim of this study was to isolate and characterize some apoptosis-related genes from *Penaeus monodon* infected with WSSV so that their expression and interaction with other proteins could be followed during the course of viral infections. First, total RNA from hemocytes of WSSV infected shrimp was isolated and used for cDNA synthesis. In addition, specific primers were designed from conserved regions of potential PCD genes of interest and used for RT-PCR. The amplified RT-PCR products were cloned and nucleotide sequences were determined followed by homology comparisons at GenBank. The full-length of selected genes was obtained by the RACE technique. Genes for 3 proteins involved in PCD were identified. These were translational controlled tumor protein (TCTP), ALG2 - interacting protein X (Alix) and inhibitor of apoptosis protein (IAP). These genes are being used as bait in a yeast two-hybrid system with a shrimp cDNA library as prey in order to screen for other PCD related proteins.

**CHALLENGING SUCCESSIVE MOSQUITO GENERATIONS WITH A DENSONUCLEOSIS
VIRUS YIELDS PERSISTENT, INNOCUOUS INFECTIONS AND PROGRESSIVE
IMPROVEMENTS IN SURVIVAL**

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Research on cultivated shrimp suggests that they have the capability to tolerate viral pathogens in a highly specific manner by mechanisms currently unknown. The phenomenon is difficult to study in detail because they have a generation time of 1-2 years and lack continuous cell lines. Thus, we developed a mosquito-densovirus model to examine whether similar phenomena occur in insects. Serial challenge of 5 generations with a stock densovirus (*ATHDNV*) resulted in progressive survival increases from 15% to 58%. Prevalence of *ATHDNV* infection in surviving mosquito larvae (confirmed by PCR, histology, *in situ* hybridization and transmission electron microscopy) was relatively high (e.g., 43% in F2) but they grew normally to establish each succeeding generation. At the end of 5 generations, comparison of putative amino acid sequences from genome fragments revealed a significantly higher ($p = 0.02$) estimated prevalence of defective targets in the survivor virus population ($29.7\% \pm 10.0$ SD) than in the stored viral population ($3.3\% \pm 5.8$ SD). The results paralleled those reported for serially passaged C6/36 mosquito cell cultures infected with densoviruses. There, reduced infection rates are ascribed to the production of defective interfering particles (DIP). Thus, it is possible that the presence of prior *ATHDNV* infections with a high level of DIP contributed to improved survival in our challenged F4 mosquito population. If so, it suggests that persistent viral infections in arthropods may serve in a specific, adaptive manner to reduce the incidence and severity of disease.

MOLECULAR GENETICS AND EPIDEMIOLOGY OF WHITE SPOT SYNDROME VIRUS OF SHRIMP

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White spot syndrome virus (WSSV, *Nimaviridae*) is one of the most important pathogens of cultured shrimp, more specifically in Southeast Asia. The virus emerged in the early 1990s near China and spread quickly through tide and trade in Southeast Asia to become a world-wide epizootic. WSSV is not only a notorious scourge for shrimp, but also attacks other crustaceans such as crabs and crayfish and forms a threat to aquatic crustaceans that are part of the food chain. Intervention strategies are being sought to control virus diseases in shrimp, in particular against WSSV, through sanitation, monitoring, eradication and maybe in the future vaccination. WSSV is a sole member of the virus family *Nimaviridae* and contains (depending on the isolate) a large, circular, double-stranded DNA molecule with about 180 computational open reading frames (ORF). The function of about 30 ORFs is known. Most of these either encode structural proteins or enzymes involved in nucleotide metabolism and DNA replication. The genome is further characterized by the presence of multiple homologous repeat regions (*hr*'s) with hitherto unknown function. In this review, an update will be given on the functionality of WSSV genes in terms of expression. WSSV isolates originating from Taiwan (WSSV-TW), China (WSSV-CN) and Thailand (WSSV-TH) show genotypic differences allowing the identification of each isolate. The variable loci were mapped by alignment of the genome sequence of these three WSSV isolates. These loci can be divided into deletions, differences in number of repeat units and single nucleotide polymorphic sites. The variation within these loci suggests a recent geographical spread from a common ancestor and we hypothesize that a genetic gradient exists through the natural spread of WSSV over time from Taiwan to Thailand and onwards via China, Vietnam and Cambodia. Analysis of WSSV isolates obtained along the coast of Vietnam (WSSV-VN) showed that all genotypes contained the same but unique deletion in "variable region ORF23/24" and that they are genetic intermediates between WSSV-TW and WSSV-CN on the one hand and WSSV-TH on the other. "Variable region ORF23/24 can be used as a diagnostic marker for WSSV isolates. Analysis of the non-*hr* unidirectional tandem repeats dispersed along the viral genome suggests that they are suitable as markers to study the spread of WSSV at the regional or local level or even forensics. The potential of molecular genetics to understand WSSV epidemiology and ecology is discussed.

PHYLOGENETIC ANALYSIS AND EVIDENCE OF GENETIC RECOMBINATION AMONG SIX GENOTYPES OF YELLOW HEAD COMPLEX VIRUSES FROM *Penaeus monodon*

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Yellow head virus (YHV) is an important pathogen of farmed black tiger shrimp (*Penaeus monodon*), causing rapidly accumulating mortalities in diseased ponds during the middle to late stage of grow-out. Several other related viruses have also been reported, including gill-associated virus (GAV) that was originally detected in healthy farmed *P. monodon* in Australia. In this paper, we describe phylogenetic analysis of sequences amplified from the ORF1b (replicase) and ORF3 (glycoprotein) genes of 54 YHV-complex viruses collected from healthy and diseased *P. monodon* from Australia, Mozambique, India, Thailand, Vietnam, Malaysia, Taiwan, Philippines and Indonesia. The phylogenies generated from each region indicated that the isolates clustered into six genotypes. Genotype 1 corresponded to the lineage of the original YHV isolate from Thailand and was only detected in shrimp from Thailand with yellow head disease. Genotype 2 corresponded to the lineage of the original GAV isolate and was detected in both healthy and diseased shrimp from Australia and healthy broodstock and post-larvae from Indonesia, Thailand and Malaysia. Other genotypes were detected in healthy shrimp. Genotype 3 was detected in shrimp from Vietnam, Indonesia and Thailand, genotype 4 was detected in shrimp from India, genotype 5 was detected in Malaysia, Thailand and Philippines, and genotype 6 was detected in shrimp from Mozambique. Two isolates from Indonesia clustered as genotype 3 in ORF1b and genotype 2 in ORF3, and two isolates from Vietnam clustered as genotype 3 in ORF1b and genotype 5 in ORF3, suggesting genetic recombination may occur between these genotypes. Sequence analysis of one Indonesian isolate indicated that the likely recombination cross-over locus was in the 3' region of the ORF2 gene.

PREVALENCE OF TAURA SYNDROME VIRUS (TSV) AND INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS (IHHNV) IN *Penaeus vannamei* AND OTHER AQUATIC SPECIES NATIVE TO THAILAND

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This study surveyed the prevalence of Taura syndrome virus (TSV) and infectious hypodermal and haematopoietic necrosis virus (IHHNV) in non-native, white shrimp (*Penaeus vannamei*) cultured in Thailand. The impact of these infectious agents on indigenous aquatic animals in Thailand was also assessed. Samples of larval, juvenile and adult white shrimp were collected from 3 regions of the country and screened for TSV and IHHNV using the polymerase chain reaction (PCR) technique. Viruses isolated from infected shrimp were used to determine the susceptibility to infection of some native Thai aquatic species. From 163 hatcheries larval samples screened, TSV and IHHNV infection was 3.7 and 44.2%, respectively. TSV infection was detected as high as 7.3% in shrimp samples collected from the southeastern region, followed by 5.6% for the eastern region and 4.5% for the central region. The highest rate of IHHNV infection was 55.6% for the eastern region. From 192 samples of juvenile shrimp from grow-out ponds, TSV and IHHNV percentages were 6.7% and 67.9%, respectively. The highest prevalence of IHHNV (up to 90%) was in samples collected from the lower Southern region. The highest prevalence of TSV infection (11.3%) was in shrimp from the Central region. Many aquatic species native to Thailand, e.g., black tiger shrimp (*Penaeus monodon*), speckled shrimp (*Metapenaeus monoceros*), dwarf prawn (*Macrobrachium equideus*), krill (*Acetes* sp.), mantis shrimp (*Chloridopsis immaculatus*), freshwater prawn (*Macrobrachium lanchesteri* and *M. rosenbergii*), mangrove crab (*Sesarma* sp.) and mud crab (*Scylla serrata*) were susceptible to viral extracts and died. Mortality associated with the causative agent was confirmed in most species, except the mud crab and freshwater prawn (*Macrobrachium rosenbergii*). However, viral particles were still detected in surviving animals 10 days after challenge. The results of this study will be a helpful in establishing measures for disease control and for reducing of risk of pathogen introduction with imported white shrimp broodstock.

VARIATION IN INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS ISOLATES FROM DIFFERENT AREAS IN THAILAND

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Nucleotide and putative amino acid sequences were determined for 6 infectious hypodermal and hematopoietic necrosis virus (IHHNV) isolates from different locations in Thailand (Bangkachai, Chantaburi, Padriew, Phuket, Nakhon Si Thammaraj, and Samut Sakhon). DNA extracts from all isolates were subjected to polymerase chain reaction (PCR) amplification and cloning using 10 pairs of primers that produced overlapping amplicons covering 94.2% of the IHHNV genome. Three primers (ck6, ck6a, and ck7) covered the ORF3 region that comprised 1,396 nucleotides from position 2606 to 4,001 of GenBank reference AF218266 and encoded nonstructural protein 1 (NS1 at 2,758 to 3,747 bp). ORF3 of isolates from Chantaburi, Bangkachai, and Padriew showed 99.9%, 99.5% and 99.6% nucleic acid identity to the Hawaiian isolate, respectively. A Mahachai isolate showed 95% identity to the Hawaiian isolate (lowest among all isolates) but 96.2% to a Thai isolate (AY102034) reported in 2002. The ORF3 sequence (1.4 kb) was selected for translation into NS1 putative amino acids before doing multiple alignment comparisons using IHHNV from Hawaii (GenBank AF218266) as the reference putative sequence. Using these putative amino acid sequences, IHHNV isolates from Chantaburi, Bangkachai and Padriew were identical to the Hawaiian isolate while the Mahachai isolate showed only 98.5% identity. By contrast, it showed 100% identity to the previously reported Thai isolate (AY102034). A phylogenetic tree based on nucleotide comparisons placed all the Thai isolates in the same clade as the Hawaiian isolate, except for the Mahachai isolate and the previously reported Thai isolate. A phylogenetic tree based on putative amino acid sequences resulted in a similar pattern. Multiple alignments and phylogenetic trees based on nucleotides 161 to 4001 gave similar results. Although putative amino acid differences in ORF 1 were distributed throughout, a high percentage of substitutions were found in the range of amino acids 155-195. These results support the previously reported hypothesis that IHHNV was transferred to the Americas via imported experimental stocks of Asian *Penaeus monodon* in the early 1980's. Reference to recent literature indicates that IHHNV is highly uniform in Asia and has undergone little genetic change in the 20 year interval since its introduction to the Americas. However, it is genetically more distant from IHHNV reported from Australia and Madagascar.

EPIDEMIOLOGIC ANALYSIS OF RISK FACTORS FOR WHITE SPOT DISEASE (WSD) IN BLACK TIGER SHRIMP *Penaeus monodon* CULTURE IN THAILAND

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White spot disease (WSD) is serious pandemic viral disease that is acutely fatal and causes massive losses with almost all species of commercially cultivated marine shrimp. A retrospective study on risk factors of WSD outbreaks was performed using management practices, water quality and environmental factors as parameters throughout several production cycles. The 314 production cycle records of a 70-pond farm covering the period January 1998 to April 2002 were analyzed using a logistic regression model. Over the whole study period, 42.7% of the ponds recorded WSD outbreaks and the outbreak pattern was similar each year, with a low prevalence in the first crop (February to August) and a higher prevalence in the second crop (September to January). Cloudy days were the strongest factor affecting disease occurrence. Ponds that had larger production areas, stocked older post-larvae and had a high density at harvest date tended to experience more disease. By contrast, ponds with longer preparation periods before stocking had a lower risk for WSD outbreaks.

ENHANCEMENT OF WATER AND SEDIMENT QUALITY IN AQUACULTURE PONDS BY MICROORGANISMS

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Microorganisms are extremely important in the ecology of aquaculture ponds. Photosynthetic forms produce organic matter and dissolved oxygen. Saprophytic species decompose organic matter (substrate) originating in uneaten feed, feces, and dead plankton under both aerobic and anaerobic conditions preventing it from accumulating to high concentrations. Chemotrophic bacteria oxidize ammonia, nitrite, ferrous iron, and sulfide. Microorganisms are ubiquitous and rapidly increase in abundance and physiological activity in response to added substrate. For example, when organic matter is applied to a pond, the number of decomposer microorganisms will increase exponentially. When the substrate is depleted, they will decline in number but remain as spores or resting cells. The main factors affecting their abundance and activity are warmth, adequate moisture, a suitable pH, the presence or absence of dissolved oxygen, and the quantity and quality of substrate. There usually is some imbalance in one or more of these factors if microorganisms are not functioning properly in a pond. Many shrimp and fish farmers, and especially those in Asia, apply microbial inocula and enzyme products derived from microbial cultures to ponds to enhance microbial activity and improve environmental conditions for aquaculture. Unfortunately, this practice is not supported by biological principles. If microorganisms are not functioning properly in a pond, it is more likely because environmental conditions are not favorable or the input of organic matter is so great that it cannot be decomposed without depleting the dissolved oxygen concentration at the sediment-water interface. Most studies have not shown significant improvement in water or sediment quality following applications of microbial products.

PROBIOTICS AND IMMUNOSTIMULANTS IN SHRIMP CULTURE

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Recently, there is an increasing health concern among the world population for preparation of safe food beginning at the level of raw materials. One strategic approach to address this concern in food industry is traceability under hazard awareness programs that convey information to farm folk on how quality and safety can be assured. Avoiding the use of chemicals and antibiotics in rearing farm animals is integral to such programs since there are known effects on human health from long-term use of antibiotics, for example. These can leave residues in meat and be directly consumed by humans. Organic products, either eco- or bio-products are currently preferred in the European community. One example is the use of starter cultures with probiotic properties in dairy products that have been designated as beneficial for humans. Their claimed functions include control of intestinal infections, control of serum cholesterol levels, improvement in lactose utilization by persons with lactose intolerance and anticarcinogenic activity. The same benefits have been expected from the use of probiotics with animals. Probiotic bacteria have been used since ~1950 in farm animals and ~1980 in aquaculture. However, interaction between the animal host and the probiotics is still ill defined. Moreover, there are very few recent reports on the use of probiotics and immunostimulant in aquaculture. This paper reviews the published literature and compares the different experimental strategies that have been used to study the phenomenon.

**EFFICACY OF PROBIOTICS AND IMMUNOSTIMULANTS IN
DEVELOPING RESISTANCE AGAINST LUMINOUS VIBRIOSIS IN SHRIMP**

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An experimental study was undertaken to evaluate the efficacy of commercially available probiotics and immunostimulants in enhancing shrimp (*Penaeus monodon*) survival, growth rates and resistance against luminous vibriosis (*V. harveyi*). Two different Probiotics (Prob-1 & Prob-2) and Immunostimulants (IM-1 & IM-2) were obtained from local companies. Healthy hatchery reared postlarvae (PL 20) were acclimatized to laboratory conditions for one month and then fed *ad libitum* for a period of one month with normal feed (controls) or feed containing probiotics or immunostimulants at 5 g / kg. The control and treated shrimp were then challenged with luminous *Vibrio harveyi* followed by estimation of growth, survival and bacterial loads at one week post challenge. There was no difference in survival of shrimp fed Prob-1 and Prob-2, but those fed Prob 2 had a higher growth rate. Shrimp fed IM-1 showed much higher survival and growth than those fed IM-2 or either of the probiotics. Survival at 1 week post *V. harveyi* challenge for the group fed IM-1 was 40%. Corresponding survivals for groups treated with Prob-1, Prob-2 and IM-2 were 20%, 6% and 20%, respectively. For the control group, mortality at 1 week post *V. harveyi* challenge was 100%. Thus, different probiotics and immunostimulants vary in efficacy for protection of shrimp against luminous vibriosis.

BACTERIOPHAGES AND THEIR ROLE IN BIOCONTROL OF PATHOGENS IN AQUACULTURE

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Bacteriophages are widely distributed in the aquatic environment and it is thought that they play an important role in maintaining the microbial balance in natural ecosystems. Bacteriophages have been found to be specific for certain microbial strains and they have been used in typing bacterial species. However, bacteriophages with a wide spectrum of activity against pathogenic strains of any bacterial species would have application in the control of pathogen populations. Management of bacterial disease in aquaculture systems has depended on the prophylactic and therapeutic use of antibiotics. However, this approach is not environmentally friendly, since antibiotic residues in foods are a major public health concern. The selection of bacterial populations resistant to antibiotics and the spread of antibiotic resistance genes in microbial populations are issues of serious concern. On the other hand, bacteriophages are found in the natural environment and therefore have a potential for application as biocontrol agents. We have isolated several bacteriophages lytic for luminous *Vibrio harveyi* from aquaculture and coastal environments. Some of the bacteriophages are lytic for the collection of *V. harveyi* strains tested and show potential for application as agents for biocontrol of this important group of shrimp larval pathogens.

EFFECTIVE CONTROL OF AMMONIA AND NITRITE IN COMMERCIAL SHRIMP PONDS USING A UNIQUE CONSORTIUM OF MICROBIAL NITRIFICATION STRAINS

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A novel consortium of microbial nitrification strains, NB700 has been developed. It has been demonstrated to be effective for ammonia and nitrite control both in the laboratory and in commercial shrimp pond studies. FISH (fluorescent *in situ* hybridization) analysis and full 16S rDNA sequence determination confirmed that the consortium consisted of *Nitrosomonas eutropha* and a *Nitrobacter* species that existed in a large flocculated biofilm structure that could be readily suspended the pond water column. Both strains had unusual tolerance to high salt and growth temperature conditions. In the laboratory and field studies with the shrimp *Litopenaeus vannamei*, periodic applications of NB700 maintained or reduced the respective ammonia and nitrite levels throughout test periods to 0.2 and 0.5 ppm or less. The pond growing period in the NB700-treated ponds could be extended to over 110 days when compared to the typical 90-day or shorter harvest time required for untreated control ponds. Yields were 15.5 and 16.6 tons/ha in duplicate treated ponds. The nitrifier consortium is compatible with a probiotic blend of PB628 *Bacillus* strains that appear to further augment good pond water quality maintenance.

SCREENING OF PROBIOTIC BACTERIA EMPLOYING GFP-TAGGED *Vibrio harveyi* IN SHRIMP LARVAE

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Probiotic bacteria for biocontrol of vibriosis were isolated from tiger shrimp larvae and the hatchery environment. Almost all isolates could inhibit the growth of pathogenic *Vibrio harveyi*. SKT-b isolate from *Skeletonema* was very effective in inhibiting *V. harveyi* and significantly reduced larval mortality in pathogen challenge assays. These prospective probiotic bacteria did not show pathogenicity to shrimp larvae at a concentration 10^6 - 10^8 CFU/ml. Partial sequencing of the 16S-rRNA gene showed that SKT-b was similar to *Vibrio alginolyticus*. To analyze the mechanism of disease suppression by SKT-b, especially with respect to competition for adhesion sites, we constructed recombinant *V. harveyi* carrying *gfp* gene as a molecular marker. *V. harveyi* carrying *gfp* cloned in a broad-host range plasmid (pWG01) resulted in green-fluorescent colonies and cells due to the production of GFP. However, under non-selective long term experiments without antibiotic pressure, pWG01 was highly unstable. Therefore, we used a mini Tn10 transposon containing *gfp* gene to yield stable transconjugants by insertion into *V. harveyi* genomic DNA. One Tn10 transformant called G3-Tn10gfp gave green-fluorescent colonies and cells. Stability analysis and pathogenicity assays showed that Tn10gfp was stably maintained in *V. harveyi* G3 strain and did not altercate its pathogenicity. In subsequent adherence assays, fluorescent G3-Tn10gfp cells could be observed inside the digestive tract of shrimp larvae and their numbers were lower with SKT-b treatment than without. This demonstrated possible competition for adherence sites or nutrition between *V. harveyi* and SKT-b.

COPPER ADDITION TO CONTROL VIRULENCE OF PATHOGENIC VIBRIO AGAINST PENAEID SHRIMP

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One of the deteriorations in aquaculture is mass mortality of shrimp caused by pathogenic microorganisms. *Vibrio* species are the most prominent pathogenic bacteria in shrimp aquaculture. Many kinds of antibiotics have been widely used to control these pathogenic bacteria. However, use of antibiotics in aquaculture may cause problems in terms of outbreak by resistant bacterial isolates. Therefore, one of the best ways to prevent problems from pathogenic bacteria is to control their virulence. In this study, we studied the effect of copper in controlling virulence of pathogenic *Vibrio* isolates. Two pathogenic *Vibrio* isolates were used in this study. Their virulence was analyzed by viable shrimp haemocyte cell counts and by MTT assay with HeLa cells using *Vibrio* culture broth supernatant prepared after 24 hours of culture. *Vibrio* challenge tests were conducted using *Penaeus japonicus*. Results from all of three methods showed that *Vibrio* toxicity was down-regulated by addition of Cu but without affecting cell growth. Moreover, it was found that copper was able to control *Vibrio* luminescence. Real-time RT-PCR assays to test for the relationship between copper level and LuxR gene expression in the form of Lux mRNA suggested that addition of copper could be useful to control the virulence of pathogenic *Vibrio*.

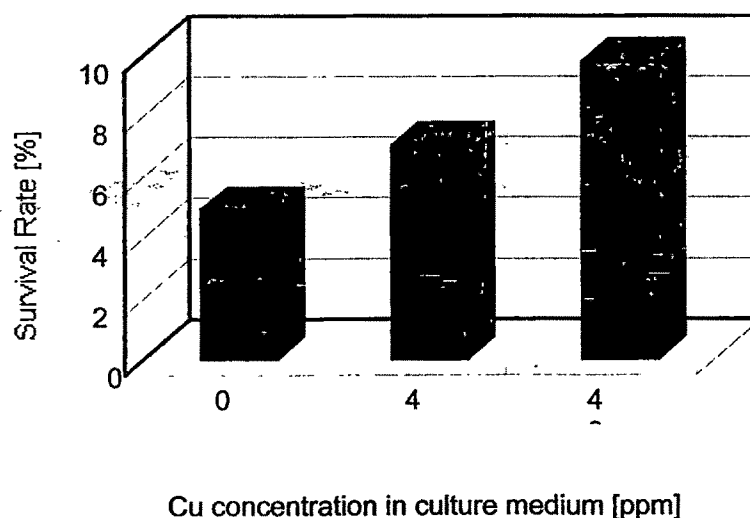


Figure 1 Survival of shrimp haemocytes 5 minutes after addition of *Vibrio* supernatant from 24-hour cultures.

THE IMMUNE PARAMETERS OF WHITE SHRIMP *Litopenaeus vannamei* EXPOSED TO COPPER SULFATE

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Copper sulfate is commonly applied to shrimp ponds to eradicate filamentous algae. It is also very effective in reducing the abundance of blue-green algae like *Oscillatoria* that synthesize and excrete aversive flavored compounds such as geosmin. Shrimp farmers often apply excess amounts of copper sulfate in pond management. Therefore, the concentration of copper sulfate remaining in the water and its effect on the resistance of cultured shrimps are of primary concern. White shrimp *Litopenaeus vannamei* exposed to 0 (control), 1, 5, 10 and 20 mg l⁻¹ Cu²⁺ for 24, 48 and 96 h were examined for THC (total haemocyte count), phenoloxidase activity, respiratory burst (release of superoxide anion), phagocytic activity and clearance efficiency to *V. alginolyticus*. Copper concentrations at 1 mg l⁻¹ or greater for 24 h resulted in decreased THC, phenoloxidase activity, phagocytic activity and clearance efficiency, whereas a copper concentration of 20 mg l⁻¹ caused a significant increase in respiratory burst. Concentrations of Cu²⁺ at 1 mg l⁻¹ or greater increased the susceptibility of *L. vannamei* to *V. alginolyticus* infection by depression in immune ability.

**BACTERIOLOGICAL, LIGHT, AND SCANNING ELECTRON MICROSCOPE STUDIES ON
VIBRIO SPECIES IN *Penaeus indicus***

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The present study describes the histopathological changes by light microscopy in the hepatopancreas (HP) of the white shrimp, *Penaeus indicus* samples from hyper saline water of the Red Sea. *In vitro* studies on the effects of both carbenicillin and *Bacillus subtilis* (ATCC 6633) filtrate on isolated *Vibrio* spp. were also investigated by scanning electron microscopy (SEM). The HP and intestine of 15 white shrimp (3-6 g) were fixed in Davidson's fixative and processed for light microscopy with H&E and Gram staining. Bacteriological isolation, counts and identification were also carried out. Light microscopy showed corrugation and separation of tubule epithelial cells and slight, moderate and diffuse necrosis. Melanized nodules were not observed. Gram-negative bacteria were identified inside the lumen of the HP tubules. Large numbers of Gram-negative bacteria in the tubules was associated with lower numbers in the intestinal lumen. Necrotic changes were attributed to the effects of isolated vibrios (*V. parahaemolyticus*, *V. anguillarum*, *V. splendidus*; *V. alginolyticus*). SEM showed that *B. subtilis* filtrate exerted diffuse damage of the outer cell wall of isolated vibrios while the antibiotic caused localized deep defects in the cell wall. *B. subtilis* filtrate also resulted in a marked decrease in the number of bacteria and a wide zone of inhibition on TCBS agar. The study showed that histopathological changes were due to septicemia from isolated pathogenic *Vibrio* species and suggested that overwhelming pathogenic *Vibrio* may decrease the intestinal flora. *Bacillus subtilis* filtrate caused more alterations to isolated *Vibrio* species than antibiotic.

GROWTH PERFORMANCE OF *Penaeus monodon* GROWN UNDER ZERO WATER EXCHANGE AND ADJUSTED CARBON TO NITROGEN RATIO

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In intensive prawn culture, ammonium and nitrite may accumulate to toxic levels under aerated conditions and water exchange is used to flush out those toxic components. However, this method of reducing nitrogenous compounds reduces biosecurity and effluent control. This work investigated survival, growth and food conversion ratio of *P. monodon* (average weight 3.34 ± 0.01 g) grown under conditions of zero-water exchange and adjusted carbon to nitrogen ratio (C:N ratio from 6.5 to 22.5) using molasses as an organic carbon source. Increased C:N ratios resulted in better growth rates and FCRs (Table 1). The better prawn performance was correlated with decreases in ammonia and nitrite levels (Table 1) and the number of heterotrophic bacteria in the culture medium. The results suggested that inorganic nitrogenous compounds were immobilised by bacteria that in turn were used as a supplement food source resulting in improved FCRs.

Table 1 Effect of C:N ratio on ammonia and nitrite in the culture medium and growth rate and feed conversion ratio for *Penaeus monodon*.

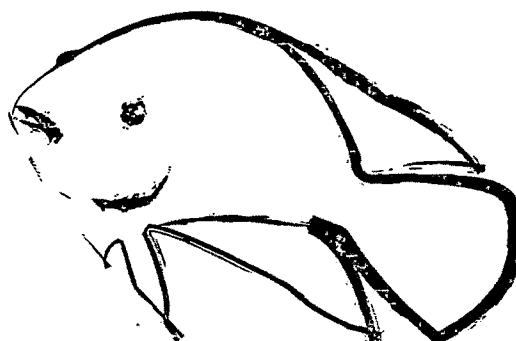
Treatment (C:N ratio)	Ammonia (mg/l)	Nitrite (mg/l)	Growth rate (g/day)	Feed conversion ratio (FCR)
6.5 (control)	$1.06 \pm 0.015a$	$45.07 \pm 0.03a$	$0.133 \pm 0.035a$	$3.398 \pm 0.257a$
15.0	$0.348 \pm 0.049b$	$2.86 \pm 0.02b$	$0.154 \pm 0.004b$	$2.889 \pm 0.070b$
17.5	$0.92 \pm 0.038c$	$0.144 \pm 0.04c$	$0.161 \pm 0.004c$	$2.715 \pm 0.087c$
20.0	$0.08 \pm 0.018d$	$0.078 \pm 0.013d$	$0.198 \pm 0.003d$	$2.216 \pm 0.036d$
22.5	$0.08 \pm 0.01d$	$0.049 \pm 0.004d$	$0.172 \pm 0.004e$	$2.388 \pm 0.118e$

SOURCE OF SALMONELLAE CONTAMINATION IN SHRIMP AQUACULTURE PRODUCTION IN WEST JAVA, INDONESIA

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Shrimp continues to be an important export commodity for Indonesia and contributes significantly to the country's revenue. However, shrimp exports have been frequently rejected due to filth, *Salmonella* and unsanitary conditions. It was previously reported that 12.5% of fresh aquaculture shrimp contained Salmonellae. This study was conducted to identify the source for *Salmonella* contamination during production of aquaculture shrimp in West Java, Indonesia. To achieve the objective, several ponds were investigated using samples of water, sediment, feed and Tiger shrimp to assay for the presence of *Salmonella* according to the Bacteriological Analytical Method (AOAC, 1995) and with confirmation by serology. Preliminary results indicated that water was the primary source of *Salmonella* contamination. *Salmonella species* (serogroup 1A), *S. arizonae* and *S. arizonae* (serogroup 3A) were the dominant serotypes found at the production level. However, additional contamination sometimes occurred during collection prior to transportation to shrimp processors. *S. choleraesuis*, presumably originated from farm animals nearby and

SS Advances in Seaweed Cultivation and Utilization in Asia



DEVELOPMENT OF THE SEAWEED CULTIVATION AND INDUSTRY IN JAPAN

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The total seaweed production in Japan from natural beds and from cultivation grounds was estimated to be approximately 650,000 tons wet wt. annually. Of this the production of cultivated seaweeds was about 615,000 tons wet wt. (94.6%). The culture of the edible red seaweed, *Porphyra*, in Japan has been established since ca. 500 years ago. The cultivation technology for the other edible seaweeds, i.e. species of the green alga *Monostroma*, and the brown algae, *Undaria* and *Laminaria*, was developed only some 40 years ago. Production of other species, such as the brown algae *Nemacystis decipiens* and *Cladosiphon okamuranus* and the red alga *Meristotheca papulosa*, have increased as seaweed salad since 1990s. The total annual income from the production of seaweeds in Japan is approximately US\$500 million. About 56,000 tons of seaweeds are imported annually for the material of food processing, agar, carrageenan and alginic acid, which are collectively worth US\$113 million.

Seaweed cultivation methods in Japan are generally classified into two types: the net method (usually 2 m x 20 m with a mesh size of 15 cm) is used for species of *Porphyra*, *Monostroma*, *Enteromorpha* and *Cladosiphon*, and the rope method, which is used for the cultivation of *Laminaria* and *Undaria*. The technology of seaweed cultivation in Japan has succeeded in increasing production of each species. Recently, the main areas in seaweed cultivation are on the genetics and disease management for production of good quality seaweeds. Japanese people have used seaweeds as food for many years. The quality of the materials for food processing is important as higher quality products are required. Nowadays the processing of *Porphyra*, *Undaria*, and *Laminaria* has become automated in large-scale factories to meet the increasing demand for these seaweeds.

The agar industry in Japan began about 400 years ago, and grew to be the biggest producer of agar in the world in the 1970s. At present, the agar produced in Japan is based on imported materials of *Gracilaria* and *Gelidium* from the other countries. The new applications of agar are based on many kinds of food and cosmetics. Alginate in Japan has been extracted from the brown seaweeds, *Ecklonia* and *Durvillea* from Chile. The alginate produced in Japan is of high quality, and is generally preferred for use in various fields. The annual production of alginate is estimated at about 1,000-1,500 tons annually. The new applications of alginate are linked to the areas of food, medicine and industrial products. Recently, there are many products of pharmaceutical functions with water-soluble dietary fibers of seaweeds (fucoidan, agar-oligosaccharide and alginic acid, etc.) in Japan. Main products are health drinks and anti-sunburn cosmetics. The three types of fucoidan brown seaweeds have three activities; the self-destruction (Apoptosis) of cancer cells, biological restoration and immunity activity. The research on experimental animals shows that certain algal acidic polysaccharides prevent the proliferation of implanted cancer cells, possibly by enhancing the immune system. In Japan, the new products with pharmaceutical functions will be promoted widely in the near future.

SEAWEED INDUSTRY OF THE PHILIPPINES

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Among the major aquaculture commodities produced in the Philippines, seaweeds contribute 66.9% of the total production, followed by milkfish (17.3%), tilapia (9.1%), shrimps and prawns (2.8%) and others (3.8%). However, export volume and value of seaweeds rank only number three next to tuna and shrimps.

The seaweed industry of the Philippines primarily involves *Kappaphycus* (*cottonii* type) farming, trading, exporting and processing. The farming and gathering of *Eucheuma*, *Gracilaria*, *Caulerpa* and *Sargassum* are only secondary.

Kappaphycus farming has positively transformed the lives of thousands of coastal families since its introduction in the early 70's. It has therefore contributed high revenue to the national economy, as well. Today, the Philippines are the world's major producer of *Kappaphycus* and also a major player in the production of semi-refined and refined carrageenan in the world. Despite the success of *Kappaphycus* industry in the country, it is still confronted with problems, the solutions to which require the interplay of the private sector, government agencies, research institutions and the academe.

On the other hand, there is a need to maximize the potential of *Eucheuma*, *Gracilaria*, *Caulerpa*, *Sargassum* and other emerging economically important seaweeds in order to address the challenges of a globally competitive market.

The seaweed industry of the Philippines has definitely established a niche in the global market and will remain one of the top, marine-based dollar-earners of the country in the years to come.

SEAWEED INDUSTRY IN THAILAND

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In Thailand, seaweeds have been traditionally utilized as human food, although it is limited to only those people living in the coastal areas along the Gulf of Thailand and the Andaman Sea. Today, seaweed has become a very versatile product widely used for food in direct human and marine animal consumption. It is also an ingredient for food and cosmetics industries and is used as an animal feed additive and for wastewater treatment. Seaweeds are harvested by hand from the wild crop population but are now increasingly cultivated. They fall into three broad groups based on pigmentation; red, green and brown seaweed. The utilized marine algae belong to the genera *Gracilaria*, *Porphyra*, *Eucheuma* (*Kappaphycus*), *Caulerpa*, *Ulva*, *Enteromorpha*, *Acanthophora*, *Hypnea*, and *Sargassum*. Of which, the red alga *Gracilaria* is the most important economic seaweed in Thailand. They are used in agar production and abalone feed. According to the report of the Thai Customs Department (1994-1996), total annual value of exported dried agarophyte is estimated at almost THB 31.4 million while total annual imported is about THB 354.6 million of processed seaweed products.

The seaweed industry in Thailand is at an initial stage. Although, cultivation of seaweed has developed since 1986, it is not popular in Thailand. The seaweed culture is mostly emphasized on the genus *Gracilaria*. Cultivation of *Gracilaria* has recently been developed in the southern provinces where the algae also occur in natural populations. Common and simple methods used for cultivation are pond culture and monoline culture. Pond culture is successful with *G. fisheri* and *G. tenuistipitata* while monoline culture of *G. fisheri* provides acceptable production.

Recently, several species of marine algae have been considered as part of a recycling treatment of wastewater from shrimp ponds. *Gracilaria fisheri* and *G. tenuistipitata* has been grown in earthen ponds using shrimp farm effluent. In addition, *Caulerpa lentillifera*, *Acanthophora spicifera*, and *Solieria robusta* are applied for wastewater treatment. Industry of seaweed culture in Thailand is being continuously developed. The focus is on management of cultivation system, because mass production and quality of seaweed materials depend on appropriate management of the culture system. Consequently, mass culture for *Gracilaria* production has developed using marine animal effluents under semi-enclosed/controlled culture system. The semi-enclosed/controlled culture system is developed from the basic concept of a balanced management of environmental factors in the culture system. This system will contribute to the sustainable development of seaweed cultivation and industry of the country in future.

SEAWEEDS FROM MANADO BAY, INDONESIA

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Since many centuries ago, macroalgae have been consumed by humans, especially in China and Japan, and have become popular foodstuffs in other Asian countries recently. In Indonesia, there is no available information on when the macroalgae started to be used as food. Even now, macroalgae are less popular than fish or shrimp as seafood. World demand for macroalgae is expected to keep on increasing due to improvement of processing technology and increased utilization. However, people in Indonesia are still not aware of the macroalgae potential.

Information on algae in Manado Bay is still lacking, particularly on their morphological descriptions which are important for taxonomic studies. It is necessary to conduct substantial studies on algal biodiversity. This is important for the management of the existing algal resources in Manado Bay.

The objectives of the study were to identify and morphologically describe all species of algae found in the study sites. This study was expected to provide the latest information regarding the species diversity potential for the sustainable and well-defined management of the exploitation of marine resources.

This study was conducted in Manado Bay (3 stations) Bunaken Island (4 stations), Manado Tua Island (2 stations), Nain Island (2 stations). Samples were collected in 3 m depth, 10 m depth and in the reef by SCUBA in each station. The research succeeded in identified 4 family, 16 genera, 41 species of Chlorophyta ; 4 family, 6 genera, 20 species of Phaeophyta ; and 8 family, 21 genera, 48 species of Rhodophyta. Hundreds specimens, however, have not been identified.

Some of the species found are important species already exploited for economic purposes in other countries. *Eucheuma* and *Kappaphycus* are the only two genera locally exploited in this area.

THE SEAWEED INDUSTRY IN SABAH, EAST MALAYSIA

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Seaweed cultivation was introduced in Sabah since 1978 and has increasingly become an economically important natural resource for Malaysia, particularly for Sabah. It is known to have wide application potentials similar to or even better than other commodities such as cocoa and palm oil. Two significant seaweed-based industries have started and are developing at a moderate pace. These are seaweed cultivation or farming and the production of semi-refined carrageenan from seaweed. Besides the government agencies focusing on socioeconomic development for rural people, there are private local companies venturing into seaweed processing and cultivation at larger scale in Semporna. Three methods of seaweed cultivation have been widely practiced and established in Sabah namely, raft system (MKII), stake system and long line system. The long line system is being widely practiced with approximately 95 % of seaweed farmers employing this method. This was due to the fact that the long line system is more economical and practically easy to handle compared to other systems. *Kappaphycus alvarezii* is the most common species and widely farmed in Sabah besides *Eucheima spinosom* in small percentage. Sabah is the sole producer of seaweeds in Malaysia. The GDP in the year 2002 was RM21.16 billion whereby fisheries sector contributed 3.2 % of the total GDP. Total production of dried seaweed in 2002 was 2,562.49 MT with the total wholesale value of approximately RM 4.4 million. Total export for dried seaweed from Sabah in 2002 was 1,750 MT by weight and RM14 million by value. Most of the dried seaweed production in 2002 was exported to the United Kingdom (65.5%), Chile (13.0%), Korea (8.0%), Japan (6.0%) and other countries (7.5 %). The main issues and challenges facing the seaweed industry in Sabah in particular and in Malaysia in general will be discussed in this paper. Major programmes in place to boost the seaweed production in Sabah, subsequently to meet projected production of seaweed in Malaysia in the year 2010, will also be highlighted.

SEAWEED RESOURCES FOR CULTIVATION AND UTILIZATION IN MALAYSIA

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Malaysia's long coastline of 4800 kilometers with sandy and muddy beaches, estuaries and mangrove swamps and solitary or clustered islands with fringing reefs provide substrates and habitats for various seaweeds. Some of these seaweeds have been or are utilized for various purposes. A study on the seaweed resources of Malaysia for cultivation and utilization, with particular focus on *Gracilaria* species has been going on for the last three years. Some of the results from that study are discussed here.

In a survey of Peninsular Malaysia and parts of Sabah and Sarawak in east Malaysia, nine species of *Gracilaria* were found. Of these, eight were terete species namely *Gracilaria manilaensis*, *G. salicornia*, *G. changii*, *G. edulis*, *G. heteroclada*, *G. fisheri*, *Gracilaria* sp. 1 and *Gracilaria* sp. 2. A single flabellate species, *G. textorii* was found. *Gracilaria* species were more diverse and abundant in the southern tip and west coast of Peninsular Malaysia than in the east coast. Most of the *Gracilaria* species collected were in mangrove or estuarine areas either attached on rocky substrate or on mangrove roots and on nets used for aquaculture in these areas. The *Gracilaria* species collected from estuarine and coral reef areas appear to be different. Biomass was relatively higher in estuarine areas. *Gracilaria edulis* is the most widely distributed species followed by *G. changii*, however, *G. changii* appears to be the most abundant species. It is mostly *G. changii* and to a much lesser extent, *G. manilaensis* that is used in some localities as a salad. Some use of *Gracilaria* as abalone feed after culture in shrimp ponds was reportedly done in 1970-1980.

In terms of agar yield and physical quality, the *Gracilaria* species with the most potential as commercial agar sources for cultivation are *G. manilaensis*, *G. changii* and *G. fisheri*. Some of the agar yields obtained were the highest recorded in Malaysia. All three species had the best agar quality when alkali treatment with 5% NaOH for 1 hour was done at 80-90°C.

In terms of growth, growing the *Gracilaria* species in tanks in the hatchery was difficult with bleaching and fragmentation the most common problems. It appears that light intensity exceeded the optimum levels for the *Gracilaria* species. In pond cultivation trials, epiphytes were the most dominant problem. In net cage culture in an estuary, grazing was the major problem. Larger scale cultivation trials in mud ponds in Johore and by spore attachment method on different substrates in Sandakan, Sabah are currently being done.

The seaweeds currently cultivated and utilized commercially on a large scale in Malaysia are the red seaweeds *Kappaphycus alvarezii* and *Eucheuma denticulatum* in Sabah. These are used as sources of carrageenan for food and non-food uses. Some are prepared as bottled pickled seaweeds by wives of fishermen. It is also available as dried seaweed packaged nicely and sold as "Eucheuma – vegetarian health food" in tourist shops in Kota Kinabalu. Cheaper dried forms of these seaweeds are sold in the local market for preparation as a sweet dessert.

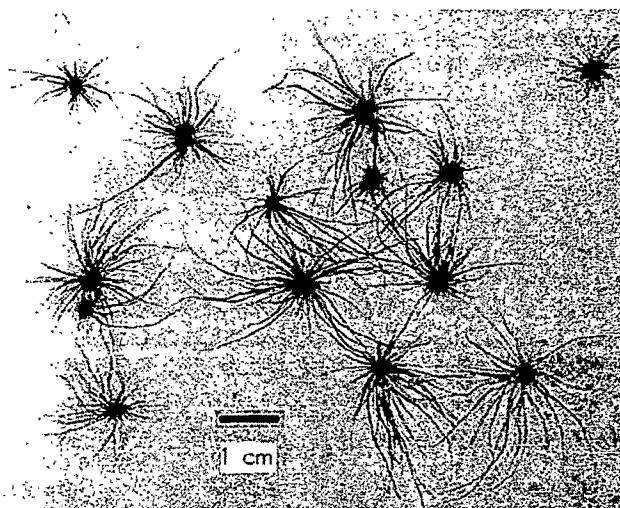
The green seaweed, *Caulerpa*, together with *Kappaphycus alvarezii* and *Eucheuma denticulatum* which are also sold in the local wet markets in Semporna, Tawau, Sandakan and Kota Kinabalu in Sabah. The *Caulerpa* was collected from Semporna. The red seaweed, *Soliera* is also harvested from natural populations in Sandakan, Sabah and sold fresh in the local market to be prepared as a salad.

A NEW TANK MARICULTURE SYSTEM OF SEAWEEDS USING DEEP SEAWATER IN JAPAN

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Wild stocks of edible seaweeds have decreased due to water pollution or overfishing in Japan. We need to establish the stable mariculture of the seaweeds. Recently, we proposed a new "germling cluster method" for tank cultivation of the seaweeds in a free-floating form. This method connected rhizoids of several seaweed germlings in the laboratory (Fig. 1) which could be cultured in a free-floating form in the outdoor tanks. Furthermore, by using deep seawater (DSW) from a depth of about 300 m with constant salinity and temperature, inorganic nutrient richness and clarity, we have designed a new tank mariculture system. On the basis of the designed system, a large scale plant for mariculture of seaweeds was first built in Muroto, Kochi in Japan on June 2004. The plant is equipped with 60 tanks of 8 ton capacity, 6 tanks of 1.5 ton and a laboratory for producing numerous *Enteromorpha* germling clusters, and supplied with 1500 ton of DSW per day. Edible green seaweed *Enteromorpha* is produced in the plant the year round. Natural populations of *Enteromorpha* occur during winter to spring but matured reproductively in high water temperature ($> 20^{\circ}\text{C}$) and disappear during the summer and autumn. *Enteromorpha* in the plant can grow well even in summer and autumn because the DSW is constantly about 12°C throughout the year. The average daily growth rate in *Enteromorpha* is 40-50%. The total annual biomass of this alga is estimated to be 3 ton dry weight from a total of about 500 ton total capacity tanks. These cultivated materials without contamination have high commercial value in the food market in Japan. It is planned to produce other seaweeds (*Undaria*, *Laminaria* and *Porphyra*, etc) as healthy food by the same mariculture system.

Fig. 1. Germling cluster of *Enteromorpha*.



ADVANCE OF *Porphyra* AQUACULTURE IN CHINA

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Development of *Porphyra* aquaculture and its application in bioremediation in China over the past 10 years is reviewed. *Porphyra* is the most valuable edible seaweed with an annual value of over \$1.5 billion. In the 1960's, China established the artificial cultivation technology for *Porphyra*. The advances made in *Porphyra* cultivation technology including selection of good strains, improvement of breeding, cultivation technology, freezing nets technology, harvest and processing, and quality evaluation in China are briefly discussed. *P. haitanensis* and *P. yezoensis* are the two main taxa cultured in China. With biotechnology, the rapid *Porphyra* breeding and strain selection with cell engineering has been established. Several good strains were selected and bred, and their yields could increase by 15-30%. Two new breeding methods (without shells) have been developed, and one of the new methods, the breeding with free-living conchospore, would be applied in *Porphyra* aquaculture in China soon. Presently the commercial cultivation of *Porphyra* in semi-floating system is practiced, and the floating system is in the process of being developed. The freezing-net technology of *Porphyra* was introduced from Japan and has been applied to prevent the diseases and green glass, and increase commercial quality. Mechanization of commercial blade harvest was just developed and it would replace the tough handwork. Most commercial blades were produced with full-automatic processing machines. Now it is possible to produce the full-automatic processing machines in China and are exported to other countries. The total cultivation area of *Porphyra* in China is approximately 23,300 ha and the total yield of *Porphyra* is about 45,000 tons (dry weight) in 2003. The yield of commercial *Porphyra* in China is the highest in the world since 2002. Our estimates of the annual uptake of nitrogen and phosphorus by *Porphyra* aquaculture are about 2,250 tons and 450 tons respectively. Hence, the seaweed cultivation is the best way to bioremediate the marine eutrophication. The coastal ecosystem of China is experiencing chronic problems due to eutrophication due to increasing population pressure along the coastal zone, as well as increases in finfish aquaculture. The seaweed cultivation would be greatly increased and extended in China, for improving the water quality and sustainable development of aquaculture.

DISEASES IN CULTIVATED SEAWEEDS IN THE PHILIPPINES: IS IT AN ISSUE AMONG SEAWEED INDUSTRY PLAYERS?

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Much of our knowledge about seaweed diseases comes mainly from those observed among cultivated species in Asia, especially species of *Porphyra*, *Gracilaria*, *Undaria* and *Laminaria*. In the Philippines, the ice-ice disease has affected the cultivated species of *Eucheuma* and *Kappaphycus*, and remained to be the most devastating causative factor up to now, even wiping out entire farms during outbreaks. Yet the issue of crop management to produce good harvest seems to have passed the attention of seaweed growers and processors. The main reason is probably due to too much attention being given to post-harvest quality improvement of the raw materials rather than with on-site cultivation. Over the years, the Philippines produce more than 80% of the world's supply of *Eucheuma* and *Kappaphycus* for carrageenan extraction. With a price per kilogram of between PhP30 - 40 (US\$0.53 - 0.71), seaweed demand is at an all time high. But while this is the case, the supply of raw materials, both for the domestic and foreign markets, remains unstable. One of the factors causing this instability can be traced to the traditional monoline technique of cultivating eucheumatoid algae, which up to now remains to be the choice of most farmers, and if there are modifications that have ever been made in some places, their success failed to attract wide application, either because they are not cost-effective for any small farm operator, or the new method is short-lived because, sooner or later, their maintenance is rendered impractical. The result is that the problem of ice-ice disease seemed to have worsened in more recent years, even to newly established farms. Now, reports of chronic cases of infestation with epiphytes and parasites (secondary infection?) are emerging problems on *Eucheuma* farming in the Philippines.

The circumstances behind the occurrence of ice-ice disease and that of epiphyte infestations are herewith described both within the context of their respective etiology. Suggestions on how to improve crop quality will be discussed in the context of seaweed health management.

ECO-MANAGEMENT OF SEAWEED RESOURCES

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Seaweeds form a very important part of the marine ecosystem. They can physically dominate the whole benthic environment and provide not only the primary food sources but also habitats for a whole range of other marine organisms. Early exploitation of seaweed resources depended on seaweed materials cast on shores, or harvesting of seaweeds from their beds at low tide. More recently, extensive farming or cultivation of seaweeds, both in the field, in ponds or in tanks, has become far more important as a way to provide the raw materials for the seaweed industries. Understanding the ecological and physiological requirements of seaweeds is therefore essential for seaweed farming to succeed. No doubt many studies have been carried out in this regard. However, the environmental effects of harvesting and farming have received much less attention. Areas need to be considered include sustainable harvesting strategies, environmental consequences of mono-culture or farming of single species of seaweed over extensive area and introduction of seaweed species for farming into areas beyond the known biogeographical range of the species. Very little information is available on any of these areas and it would help to manage our seaweed resources better if we pay more attention to them.

CROP IMPROVEMENT IN SEAWEED CULTIVATION

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Presently, seaweeds are used as human food, feed, fertilizers and as sources of industrial gums and chemicals. Seaweed cultivation is developed to meet the demands for commercially important seaweeds such as *Laminaria*, *Undaria* (brown algae); *Porphyra*, *Gracilaria*, *Kappaphycus*, *Eucheuma* (red algae) and *Ulva* (green algae). Several techniques have been developed to improve the management of seaweed cultivation such as improved harvesting methods, reduced interspecies competition and the use of artificial structures such as ropes and other substrata. Conventional plant breeding and strain selection utilize the spontaneous or induced variation in cultured cells or tissues. More recently protoplast fusion has been shown to be able to produce improved strains. Efficient seeding, increase in growth rate and genetic improvement are alternative ways to optimize the exploitation of these seaweeds. Successful seaweed transformation has been reported for *Laminaria*, a temperate brown seaweed, from China. Crop improvement through genetic transformation will be discussed in this paper, with focus on the tropical seaweed, *Gracilaria changii*.

MOLECULAR TAXONOMY OF SEAWEEDS WITH EMPHASIS ON RHODOPHYTA AND PHAEOPHYTA

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Despite the long history of taxonomic studies of seaweeds, the identification and species delineation of seaweeds is still problematic. This is due to lack of distinct morphological characteristics and the plasticity of the seaweeds. The advancement and new discoveries in biochemistry and molecular biology has helped greatly in the identification of seaweeds by the use of nucleic acids in seaweed systematic. The correct identification of the potentially commercialisable taxon or species for agriculture is very important. This paper will discuss the advancement of molecular techniques for the taxonomic and phylogenetic studies of seaweeds. The use of molecular taxonomy in tropical seaweeds based on the Malaysian *Gracilaria* and *Sargassum* as examples will be discussed.

FUNCTIONAL GENOMIC RESEARCH OF *Gracilaria changii* A TROPICAL SEAWEED

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Seaweeds are valuable sources of food, biochemicals and pharmaceuticals. However, they remain one of the least-studied groups of organism in spite of the rapid pace of acceleration in genomic research. The total number of genomic investigations of seaweed is scarce despite their ecological and economic importance. Thus, there is an urgent need to expand the corpus of knowledge in genetics, molecular biology and biochemistry of these organisms that are potentially important as a new emerging source of novel genes, and unique biosynthetic pathways and enzymes.

Large-scale partial sequencing of randomly selected cDNA clones or the Expressed Sequence Tag (EST) approach is a powerful tool for gene discovery and characterization in seaweeds where little genomic information is available. The large-scale generation and sequencing of ESTs from *Porphyra yezoensis* has been proven to be an effective approach to isolate and characterize new genes from seaweed. In Malaysia, an EST project on *Gracilaria changii*, an economically important indigenous red seaweed, was initiated to facilitate the transcriptomic research of this agar-producing seaweed.

To date, a total of more than 5000 ESTs have been sequenced and clustered into contigs, of which approximately 75% showed low similarity to other genes in the database, justifying the potential of seaweed as a source for novel gene discoveries. Among the 25% contigs that had significant similarity to the genes in the database, there were ESTs corresponding to diverse functional groups e.g. metabolism, protein synthesis, transcription, transportation, protein destination and storage, energy, intercellular trafficking, disease and defense, cell growth and division, signal transduction; reflecting the transcriptome of this tropical seaweed.

The cDNA clones generated from this project were immobilized on cDNA microarray slides to facilitate the study of gene expression profiles of *G. changii* of various physiological states. The transcriptome of *G. changii* in response to salinity, light intensity, pH and other factors that influence its cultivation and utilization, will be investigated to shed some lights to its gene functions for future genetic manipulation and species improvement.

QUALITY CONTROL OF ECONOMIC SEAWEEDS (WITH PARTICULAR REFERENCE TO CARRAGEENOPHYTES)

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The past 40 years have seen a marked transition in the supply of raw materials for the colloid industry, in particular for sources of the hydrocolloid carrageenan, where there has been a revolutionary change from the collection of materials from wild populations to the predominance of the supply of raw material coming from cultivated materials – true marine agronomy! However, cultivated materials cannot supply all of the colloidal properties required and a sustained demand exists for materials from wild, harvested populations.

This paper will expand on the concept of raw material security – not only the responsibility for security of supply of materials of acceptable quality from the farmers/traders to the factory, but also the responsibility of the hydrocolloid producer to the client, the end user of the colloidal properties.

The generalized supply chain of seaweeds from the farmer to the processing factory will be discussed. There is an obvious need for security of supply to large factories with continuous production. Any products for such a sophisticated market such as hydrocolloids must also be subject to strictly applied and well-defined quality control criteria. This is particularly true if the products are intended for pharmaceutical applications where traceability of the material is paramount.

Quality control for seaweeds is a difficult concept to apply – Seaweeds are “natural” products and so a large degree of inherent variation can be expected in their qualities. One can expect the variations to be larger in raw materials which are still collected from wild populations. In contrast, cultivated materials are generally more homogeneous; as can be expected, seasonal and geographic differences are evident in both wild and cultivated seaweeds. Some selected aspects of farming practice apparent environmental change and impacts on quality will be discussed. These problems of variation may be further exacerbated in that the raw materials are generally purchased on the basis of bulk biomass and supplied by the container load. The general quality criteria applied are often those which can be simply assessed in the field such as moisture content and presence of foreign material. Until now, it has been difficult to differentiate the quality and yield of the carrageenan present in the raw material.

In marked contrast to the bulk purchases of seaweed raw materials, the hydrocolloids are speciality additives, often applied in proprietary blends at relatively low concentrations, to be used in processed foods and many other applications, where specific, predictable and reliable rheological properties are required and the “consumer” (e.g. food manufacturer) places a value on this as is reflected in the price paid for the product and applications support.

Seaweed producers and processors have a duty of care and it is very important that perceptions of “quality” are dealt with in an open and transparent manner. Recent concerns about seaweed quality have included SEM (semicarbazide) and heavy metals (e.g. arsenic/arsenates) and issues of genetic modification. These points contributing to overall quality control of seaweeds for carrageenan will be summarized during the presentation.

It is perhaps time that seaweed “crops” were differentiated in their value based on the content and quality of pure hydrocolloid which they contain and not merely their bulk biomass. All seaweeds are created equal – but some are more valuable than others!

**CARRAGEENAN PRODUCTION BY TACARA SDN. BHD. IN TAWAU, SABAH,
MALAYSIA**

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TACARA, which stands for Tawau Carrageenan, started as an idea by its four founding directors. The idea and vision simply put was to enhance the value of the abundantly available local seaweed. Inspired by the vast potential and spurred on by enterprising spirit, in 1996 Tacara Sdn Bhd was incorporated with determined focus of processing seaweed (*Eucheuma cottonii* and *Eucheuma spinosium*) into carrageenan.

Today, Tacara is a manufacturing concern with a factory operating in Tawau, Sabah. Primarily, the factory produces Food Grade Natural Carrageenan powder and compounds for various food and non-food applications.

Tacara has its own well-equipped laboratory at its manufacturing plant where most of its research and development are conducted. Research and development of certain innovative development are conducted in collaboration with research institutions.

Tacara also has its own seaweed farm for production of its own supply of raw material. Tacara's commitment to the development of this industry involves both up and down stream processes.

Tacara began as an idea. To date it is producing and exporting good quality Food Grade Natural Carrageenan. It is where it is because of hard work of its dedicated staff and generous support of its customers. We want to move on! We want to see our goal of becoming a leading Natural Carrageenan producer by producing world-class products and solutions come true. We realize it will not be an easy task. A journey of a thousand miles starts with a single step. We believe we have indeed started the journey not only for ourselves but also for the nation in this particular industry.

PRODUCTION OF QUALITY DRIED SEAWEED: CHALLENGES AND STRATEGIES

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Farming of carrageenophytes genus *Kappaphycus/Eucheuma* has been a practice in the South East Asian coastal waters since the early 1980s. These eucheumoids are cultivated via selection techniques for the improvement of seaweed stock and yield. After almost 25 years, various improvements have been introduced, i.e. farming techniques and strain selection, to improve production of quality dried seaweed for the carrageenan industry. However, limited information is available pertaining to research done on seaweed diseases and post harvest processing of the harvested seaweed. In Malaysia, farming of carrageenophytes is done in two districts; 1) Semporna (South-East coast of Borneo), and 2) Kudat (tip of Borneo). As part of our research and development activities at our laboratory, we looked into the dynamics of seaweed diseases and post-harvest processing of the seaweeds. This paper will first delve upon the etiology and dynamics of seaweed diseases; "ice-ice" disease and epiphyte invasion. Then, details of improved post-harvest processing technique will be discussed. Information obtained from our studies has given us in-depth knowledge pertaining to the seaweed diseases and alternative methods of processing seaweed in our effort to produce quality dried seaweed for the seaweed industry.

SS Mariculture in the Asia Pacific Region :
Recent Developments and New Challenges



AN OVERVIEW OF MARINE FINFISH AQUACULTURE IN THE ASIA-PACIFIC REGION

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Marine finfish aquaculture production in the Asia-Pacific region has grown by 240% since 1990, largely due to increased production by China. In 2002, annual aquaculture production of marine finfish in the region was around 1 million metric tonnes and valued at US\$3.2 billion (FAO data). The major producer countries were China (58%) and Japan (27%).

Species cultured range from low-value species, such as milkfish (*Chanos chanos*) to the highly valued grouper species (Serranidae, Epinephelinae). A specific sector of production is the high-value southern bluefin tuna (*Thunnus maccoyii*) which is grown-out from captured juveniles in South Australia.

Production systems range from coastal ponds (often utilizing disused shrimp ponds), coastal cages (typically small-scale family based operations) to large-scale sea cages.

Despite the diversity of species and production systems in use in the region, there are a number of common constraints to the development of sustainable marine finfish aquaculture. The first of these is the supply of juvenile fish for grow-out. Many aquaculture sectors still rely heavily on the supply of juvenile fish from the wild. A recent FAO publication discusses capture-based aquaculture industries for four groups in detail and discusses the issues relating to capture-based aquaculture sectors. Hatchery production of many species is increasing, an example being the dramatic increase in hatchery production of groupers from Indonesian hatcheries in the last 5 years.

A second major constraint is the development of compounded diets to replace the use of 'trash' fish which is widespread in the region. In many countries it is the higher priced aquaculture commodities (such as groupers and lobsters) that compete successfully for limited supplies of trash fish and are restricting the availability of trash fish for other traditional uses, such as the manufacture of fish sauce. While pellet diets are now available for a range of species, there is a need to improve the cost-effectiveness of many diets to improve farmer uptake and reduce the use of trash fish.

Regionally, the development of sustainable marine finfish aquaculture is supported by the Asia-Pacific Marine Finfish Aquaculture Network, which provides a mechanism for enhanced collaboration in research, development and extension activities and supports improved communication mechanisms for marine finfish aquaculture development. For more details, see www.enaca.org/marinefinfish.

**ENVIRONMENTAL TRENDS AND CONSTRAINTS IN MARICULTURE DEVELOPMENT
IN THE ASIA-PACIFIC REGION**

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The Asia-Pacific region is the world's biggest producer of farmed marine products, and a major exporter and market for seafarming products. Further development of marine aquaculture in the region appears to be essential to contribute to future human demand for marine products, in the face of expanding human populations and stagnant or declining wild fisheries stocks. The development of mariculture though faces substantial challenges, amongst which environmental constraints are likely to be particularly significant.

This paper reviews current trends in mariculture development in the Asia-Pacific region, with particular attention to major environmental trends and constraints. The paper identifies key environmental issues, discusses environmental management options and presents recommendations on directions for future research and development support environmentally sound development of the mariculture sector in the region.

HEALTH MANAGEMENT PRACTICES IN ASIAN MARICULTURE – CURRENT STATUS AND CHALLENGES

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Disease is a major constraint affecting profitability and development of the aquaculture industry. Intensification of aquaculture and transnational movement of animals has led to an increase in the spread of disease in Asia. In a recent alert issued by FAO (Sep. 27 2004), outbreaks of aquatic animal diseases are becoming more frequent in Asia and they have caused substantial economic losses. Infectious diseases are caused by viral, bacterial, fungal and parasitic pathogens. They are responsible for almost all the devastating mortalities in fish and shrimp farming.

Asian aquaculture is characterized by an enormous diversity of species, with several dozen marine species being farmed. Consequently, more resources are needed to understand the basic epidemiology of diseases in the various species. Most farming is operated on a small scale and technical support, such as disease diagnosis and training, is lacking. More focus is on treatment rather than prevention, and antibiotics are available and widely used. In Asia, most individual farms produce several species of fish. Trash fish are widely used as feed. Fry are often wild caught or derived from wild-caught broodstock. Furthermore, legislation for and implementation of farming licenses and zoning policies are not in place in most Asian countries. With a 'gold rush' mentality, this often results in too many fish and too many farms in a concentrated area, which in turn promotes the spread of diseases. The combination of all these factors, together with the diversity of organisms in tropical waters, leads to a truly challenging disease situation. Poor health management has two fiscal implications: loss of productivity due to animal mortality and loss of trade due to food safety issues.

Good health management is the only "silver bullet" for disease control. Collectively, it includes the use of healthy fry, optimized feeding, good husbandry techniques, disease monitoring, sanitation, vaccination and proper control and biosecurity measures when diseases do occur. Overall, emphasis must be on prevention rather than treatment. Vaccination has been proven to be a powerful tool for disease control in modern fish farming. In Asia, with the exception of Japan, no vaccines for marine fish are available yet on a commercial scale. The major advantages of vaccination over therapeutic treatments are that vaccines provide long-lasting protection and leave no adverse residues in the product or the environment.

Asia dominates global aquaculture and it has yet to reach its potential. Under the threats of disease epidemics and consumer pressure on food safety, the industry must undergo changes. In recent years, more collaborative efforts from governments, academia and private sectors are being made to promote good health management for disease control. This is the only way to keep the Asian aquaculture industry sustainable.

ESCAPES OF FARMED FISH: ECOLOGICAL AND GENETIC IMPACTS ON NATURAL POPULATIONS AND HOW TO MANAGE THEM

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Aquaculture continues to expand rapidly throughout coastal Asia. As a result, escapes of farmed fish are increasingly common, and interactions between cultured and wild fish pose new challenges for the conservation of wild stocks. Impacts of escapees on wild conspecifics are dependent on the genetic origin and domestication history of farmed fish, the relative abundance of escapees, feral and wild populations, and the ecological status of the latter. Genetic origin and domestication history influence the performance of escapees in the wild, and therefore ecological and genetic interactions. In general, farm escapees show lower survival and reproductive success than wild conspecifics and this often limits their ecological and genetic impact. Nonetheless, impacts of escapees can be very significant where their abundance is high relative to the wild population and in particular where the latter is already in decline due to overexploitation or habitat degradation. Ecological interactions may be just as important a threat to wild populations as genetic introgression, which has received far more attention in the literature. A framework for understanding and predicting farmed-wild fish interactions and the implications of alternative management measures is urgently required.

Systematic risk assessments accounting for the level of escapes, biological characteristics of escapees, and status of wild stocks should be carried out to identify aquaculture sectors in which escapes pose significant ecological risks. Where this is the case, measures should be implemented to minimize risks. The first priority must be to improve containment of farmed fish by identifying and improving husbandry conditions and practices most commonly associated with escapes. In addition, there may be opportunities to facilitate recapture of escapees through conditioning for site fidelity. Advancing domestication of farmed fish to increase their dependence on culture conditions and thus reduce performance on the wild is likely to be the most effective strategy for reducing impacts of escapees, and is also compatible with the aim of improving the performance of cultured stock. Inducing sterility through triploidisation can eliminate direct genetic interactions, but does not address ecological and indirect genetic effects which are often just as significant. As an alternative it has been argued that farming fish of local wild genotypes will reduce risks to wild populations. However, maintaining wild genotypes under farm conditions is inherently difficult and also implies foregoing the benefits of domestication for aquaculture production. No single measure can eliminate the problem of farmed-wild fish interactions entirely, but a combination of greater containment, recovery of escapees, and deliberate domestication to increase dependence on humans has the potential to reduce impacts significantly.

MARICULTURE IN THE PACIFIC

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Aquaculture is an emerging industry in the Pacific region. The vast majority of Pacific Island country and territories are coastal states and mariculture is a widely applicable. Whilst the region does not have a well-developed tradition of mariculture the "sea" is synonymous with it's natural and cultural heritage. Harnessing this acutely developed awareness is an important avenue to appropriate and sustainable forms of mariculture development. Already diverse commodities are farmed - black pearl and marine prawn dominates the value of production, whereas seaweed and marine ornamentals species are higher volume. The region has a history of both successful and failed mariculture ventures and there has been a recent rejuvenation of efforts by the private and public sector in the expectation that mariculture will be a significant industry in the near future.

**SALMON FARMING, A GLOBAL SUCCESS STORY – WITH FOCUS ON THE IMPACT
OF VACCINES ON THE NORWEGIAN INDUSTRY**

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it is exactly 30 years since a pilot salmon farm in Scotland harvested the first 34 tonnes of salmon. This industry has developed dramatically and now produces nearly 1.5 million tonnes annually, spread across the two powerhouses of salmon farming, Norway and Chile, contributing 73% between them, with the remaining 27% coming from the UK, Canada and some smaller producing countries.

In 1988, less than 50,000 tonnes of salmon were produced in Norway but more than 50,000 kg of pure antibiotics was used for disease control. Since then, salmon production has increased to more than 500,000 tonnes per annum and the use of antibiotics within the industry is virtually non-existent. Vaccination has contributed hugely to this success. All Norwegian salmon and trout are now vaccinated by injection, the vast majority with oil-based vaccines, protecting against up to five different bacterial and one viral pathogen. This year, 180 million salmon and trout juveniles will be vaccinated in Norway alone.

The first commercial vaccine was developed for the salmon farming industry in Norway about two decades ago. Over the years, vaccines have had an enormous effect on the Norwegian fish farming industry. In 1985, only 2 kg of salmon were harvested for each smolt transferred to seawater and this was mainly due to losses through diseases. In 2004, it is estimated that, for every fish transferred to sea, 3.8 kg will be harvested. An independent analyst calculated that Norway has produced 2,769,000 tonnes of salmon between 1986 and 2000; without vaccines, this figure would probably only have been 1,978,000 tonnes. The value of these extra 790,000 tonnes, based on today's prices, is nearly US\$ 3 billion.

While the success of the Norwegian salmon industry is directly associated with advances in culture techniques, feeding strategies, processing technology, marketing and general health management, vaccination has certainly played a significant role.

ECONOMIC ASPECTS OF MARINE FISH CULTURE IN THE ASIA-PACIFIC REGION

Sih-Yang Sim

Marine fish culture has taken off at a rapid pace in the last five years in the Asia-Pacific region, with breakthroughs and improvement of hatchery technologies and techniques for several marine fish species. The fingerlings of some economically important marine fish species such as grouper, snapper, threadfin, pompano, and cobia are now available through hatchery production, and the farming of these species has increased recently. Although fingerling supply from hatchery is still limited for species such as grouper, improvements in larviculture techniques will see grouper fingerling supply from hatcheries increase and reliance on wild fingerlings reduce.

Marine fish species farmed in Malaysia still rely heavily on imported fingerlings. Species such as grouper, snapper, pompano, threadfin, cobia, seabass are just a few that are found commonly cultured in marine fish farms in Malaysia.

In Thailand, most of the fingerlings of the marine fish species cultured are collected from the wild, with the exception of seabass.

In Indonesia, marine fish farming is developing rapidly. In 1999, there was only one grouper farm in Lampung with 8 units (i.e. 1 unit = 4 cages measuring about 4 x 4 m or 3 x 3 m). However, in 2004 the number accelerated to some 800 marine fish cage units (800 x 4 = 3,200 cages). The development is mainly focused on grouper species, particularly humpback (*Cromileptes altivelis*) and tiger grouper (*Epinephelus fuscoguttatus*), due to availability of fingerlings from hatchery.

Marine fish farming in Indonesia and Malaysia is a capital intensive business venture. Floating net cages are used for marine fish farming and most of the farms developed in Indonesia and Malaysia are medium - to large-scale with solid wooden structures. Big polar cages are also used for marine fish culture in Malaysia. In Thailand, marine fish farming is more low cost investment which is run by small-scale farmers. Whether it is a capital intensive business venture or small-scale low cost low input farming, the economic impacts cannot be overlooked. Employment and income generation from either large-, medium-, or small-scale marine fish farming is of vital importance, particularly for communities living near the farming areas.

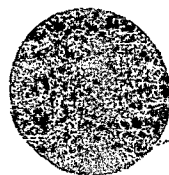
**INDUSTRY STANDARDS FOR RESPONSIBLE FISHING PRACTICES: THE LIVE REEF
FOOD FISH TRADE IN SOUTHEAST ASIA AND THE PACIFIC**

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In response to global concerns over the rapid expansion and overexploitation of the world's fisheries, new concepts which would encourage and lead to responsible, sustained fisheries are being promoted. More recently, these concerns have led to the adoption of collaborative management programs based on common principles and standards as a means to conserve fishery resources including: voluntary Codes of Conduct, certification and eco-labelling and industry standards. Among the world's fisheries, coral reef ecosystems are important to millions of people in Southeast Asia and the Indo-Pacific, as a source of food and employment. It is estimated that nearly 90% of Southeast Asia's reefs are threatened by human activities such as over-fishing and destructive fishing. The application of collaborative models to coral reef fisheries, while needed, is challenging given the high percentage of subsistence and artisanal fishing communities and the in-country capacity to implement these programs. One example is the trade in Live Reef Food Fish, which is valued at more than US\$500 million annually.

This presentation presents an overview of the trade and discusses a project that has developed voluntary standards of best-practice for the trade incorporating both wild-caught and aquaculture aspects. In particular, a proposed plan for implementing these standards is presented. It highlights the link between production, management and consumption and the need to engage stakeholders across the chain of custody from fishers to restaurants. It also addresses the unique aspects of the trade that hinder acceptance and adoption of responsible best-practices, the strengths of using regional organizations and the collaborative role of government and non-government agencies to develop and implement them.

SS Technology Needs and Prospects for Asian Aquaculture



CHANGING STRUCTURE OF FISH CONSUMPTION, PRODUCTION, AND TRADE: THE ROLE OF AQUACULTURE IN THE ASIAN DEVELOPING COUNTRIES

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There have been significant changes in the global consumption, production and trade in fish and fisheries products. The growth in exports of fish follows a tremendous growth in production and per capita consumption. The per capita consumption of fish in the developing countries was more than doubled over the past three decades, while it only increased by 1 kg in the developed countries from 22.4 kg to 23.5 kg. Along with the impressive growth in global fish production there has been a change in the composition of fish production by culture and capture fisheries. Capture fishery lost its potential to increase production. Aquaculture production from both inland and marine waters continued to grow from around 18 million tons in 1994 to 32.9 million tons in 1999. Most of the aquaculture production over the years came from Asian regions of which China is the dominant. The growth rate of aquaculture production is much higher than that of capture fishery in Asia, although the relative contribution of capture fishery production to the total fish production is still higher (62 percent) in the region. The development of aquaculture technology for both inland and marine waters and extension of the same in various intensity and practices at the farmer level has made enormous impacts on the fisheries sector of the Asian developing nations. Provision of diversified fish feed sources, supply of improved genetic strains and materials, utilization of indigenous and non traditional water areas such as rice fields, reservoirs and irrigation waters, and use of polyculture to optimize primary production played dominant role in the increase in production from culture based fishery. Systematic selective breeding has achieved significant increase in productivity for two important species, Salmon and tilapia. The paper reviews the structural changes in the fisheries sector in Asia, discusses the driving forces in the increase in supply of and demand for fish and fish products, and economic and environmental implications to the fish exporting developing nations. The paper also raises issues relating to poverty and long-term food security of the poor people in the fish exporting countries.

DEMAND FOR FISH IN ASIA: A CROSS-COUNTRY ANALYSIS

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This paper sought to establish the fish consumption pattern in nine Asian countries consisting of Bangladesh, China, India, Indonesia, Malaysia, Philippines, Sri Lanka, Thailand and Vietnam. It also aimed to estimate the price and income elasticities of fish demand by species as well as by income groups, i.e., low, middle and high-income households. A multi-stage budgeting framework was used in the analysis, which estimated food/fish expenditure function(s) in the initial stage and a system of demand equations for fish by species using a quadratic almost ideal demand system (QUAIDS) model in the final stage. Possible bias in parameter estimates of the model brought about by zero consumption of certain fish species resulting from non-preference or infrequent purchases was addressed through the use of the Heckman procedure or LIMDEP models. Results showed that price and income elasticities varied widely for all fish types investigated in the 9 countries. Additionally, all income elasticities were found to be positive. These imply that fish, in general, is a normal and a homogeneous product and a normal good. Specifically, fish was found to be more price and income elastic among the lower income groups. This suggests that the fish consumption of poorer households respond more to prices and income than the richer households. At the same time, the high-value fish types exhibit higher elasticities than the low-value species. Hence, any policy that can trigger a reduction in fish prices (e.g., increase production through aquaculture) or improvement in the livelihood of households will increase demand for fish especially among the poorer households.

FINDING NEMO: ESTIMATING IMPORT DEMAND FOR LIVE REEF FOOD FISH

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Reef fish traded alive for table food are high value-to-volume products, with demand centred in the luxury markets in Hong Kong, and southern mainland China. Approximately 15-20,000 tons of live reef fish are imported into Hong Kong, annually, valued at approximately US\$350 million. These fish are consumed in high quantities during special occasions and festivals. Approximately 20 countries in the Asia-Pacific region supply the market, which has been somewhat volatile in recent years. In this paper, import demand functions are estimated for Hong Kong/southern China, for each of the main supplying countries and fish species. Cross-price elasticities, income elasticities, and the impact of season, the Asian Economic Crisis and SARS, are estimated and the implications of the findings are discussed.

**PROJECTIONS AND SCENARIOS FOR ASIAN FISHERIES: THE ROLE OF
PRODUCTIVITY IMPROVEMENTS IN AQUACULTURE**

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The AsiaFish model is a multicommodity, well-disaggregated fish sector model incorporating production (culture and capture), consumption, and trade. It has been applied to gauge market potential of commodities as well as assess a number of policy options using simulations of the most probable as well as alternative scenarios, for the next 15-20 years. The paper discusses the model structure, data construction, parameter estimation and calibration, and simulation results. Some of the key findings are: production, consumption, and (for the most part) imports are expected to expand; production increases are dominated by aquaculture; in several countries per capita consumption is projected to fall; and the policy scenario that best increases welfare of the poor appears to be productivity improvements in low value aquaculture.

PROFILE OF BRACKISHWATER AND MARINE AQUACULTURE TECHNOLOGY IN ASIA

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Brackishwater aquaculture is becoming an increasingly important source of food fish. Asia accounted for 73.97 % of the world's total brackishwater aquaculture production. Indonesia, Thailand, China and Philippines are the top producers of brackishwater aquaculture in Asia. This paper reviews the profile of brackishwater/ marine aquaculture technology and provides a comparative analysis of production, farming practices, and economics of key farming technologies in brackishwater and marine aquaculture environments in nine Asian countries. Results show that the brackishwater pond culture is the most widespread system in terms of production, area and number of farmers in most of the countries under study, with shrimp being the most dominant species. Cost and returns vary between countries due to differences in technology, production environments, resource endowments and input cost. Shrimp farming in Malaysia is most profitable, though shrimp culture in India, Bangladesh, Vietnam and Thailand also generate good returns. In the case of finfish aquaculture, grouper culture in cage in the Philippines is most profitable.

PRIORITY OPTIONS ON TECHNOLOGY AND POLICY FOR ASIAN AQUACULTURE

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The WorldFish Center has recently completed a landmark study on strategies for increasing and sustaining production in aquaculture and fisheries to benefit poor households in Asia. This paper discusses the major findings of the paper with respect to aquaculture, including the production analysis of fish farming, the socioeconomic profile of fish farmers, pro-poor yet economically viable technologies to be prioritized in public and donor investment programs, as well as the set of options that will promote the sustained flow of benefits to the poor from aquaculture.

STRUCTURE AND PROCESSES OF INPUT MARKETS FOR AQUACULTURE IN THE PHILIPPINES: IMPLICATIONS ON AQUACULTURE'S SUSTAINABLE DEVELOPMENT

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Aquaculture in the Philippines needs efficient and sustainable sources of inputs to meet development goals. This study aims to determine the structure and processes of the input markets that serve the grow-out sector. This paper reports the results of a nationwide survey of 139 farmers that grow milkfish *Chanos chanos* (Forsk.), tilapia *Oreochromis* sp., shrimp *Penaeus monodon*, grouper *Epinephelus* sp. or mudcrab *Scylla* sp.; and 59 suppliers of inputs categorized as: construction materials and equipment; credit; feeds, fertilizers and other substances; labor; land; seedstock; technology; and utilities. Semi-competitive structure prevails for feeds and fertilizers, and seedstock markets where differentiated products are traded through geographically dispersed channels. The labor market exhibits a semi-competitive structure arising from specialization of skills. Markets for credit, utilities, and equipment are competitive. The survey results showed inequitable access to land suited for aquaculture. Complimentary relationship prevails among suppliers of technology. Comparison of inputs required vs. obtained by the grow-out operators showed competitive markets met demanded quantities. Inputs in imperfect markets were either obtained in excess or below required quantities, in both cases these are inefficient. Research and policy issues in support of the technological and institutional changes to attain efficient and sustainable input markets are discussed.

NILE TILAPIA SEED PRODUCTION IN SPRING IRRIGATED RICE-FIELDS IN NORTHWEST BANGLADESH – AN APPROACH APPROPRIATE FOR POORER FARMERS?

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Common carp (*Cyprinus carpio*) seed production in irrigated spring (*boro*) rice-fields based on stocking fertilized eggs has been promoted among farmers in northwest Bangladesh for several years. The spawning and nursery of Nile tilapia (*Oreochromis niloticus*) in similar irrigated rice-fields had been successful in northern Vietnam and demand for this species in Bangladesh led to the current trial among farmers already producing common carp. Participatory research was conducted with poorer farmers in two communities Girai (G) and Bahagili (B) to assess the appropriateness of the approach. The selected households (G=15, B=4) were practicing Integrated Pest Management (IPM). Each household allocated one rice growing plot not currently used for common carp seed production for the trial. A small number of mature GIFT strain Nile tilapia (12 female and 6 male; 100-125g size) were supplied to each trial farmer irrespective of the size of their trial plots. Management practices, production and sales were monitored weekly. The trial started in *boro* season at the beginning of 1999 and ended at the end of *amon* season in December. The average size of the plots allocated for the trial was similar in both communities (G=0.12±0.09 ha, B=0.14±0.07 ha). Each plot had one ditch with an area ranging from 20-120m². The majority of the farmers succeeded in producing fingerlings in their plot, 10% failed totally but around 70% produced more than 2000 juveniles from a single plot. The production of fingerlings by households in the two study areas was not different (4016±3350). Efficiency of production was high, averaging 17.3 juveniles.kg⁻¹ female.day⁻¹. Most fingerlings were sold (43%) or stocked for further culture in their own systems (39%), but some were used directly for household consumption (17%). Fingerlings sold were mainly purchased directly by local grow-out farmers or by fingerling traders. There was a large monthly variation in the number of fingerlings harvested but the majority of fingerlings (91% total) were harvested in April-July, peaking in the months of May-June (60% total) in both study areas. From August to December the total number of seed harvested was only 9% of the total. Fish seed made an important contribution to overall income from the trial rice plots; an average 39% of the net income was derived from fish and 61% from rice production. The tilapia seed produced were large (mean individual harvested weight =21g) and considered of high quality and available at the time of year when demand was greatest for stocking and sale.

In the trial households, women and children played active roles in management, harvest and marketing of tilapia seed. Calculated entry costs for tilapia seed production in rice-fields were low provided mature fish were supplied to the farmgate. Potentially this makes the approach attractive to poorer farmers with limited access to rice land, provided the issue of broodfish supplies and maintenance between seasons can be addressed. The sale of seed through a variety of local marketing channels also suggests that such decentralized seed supply systems are likely to result in strong multiplier effects that benefit poor intermediaries and consumers.

TOWARDS LOCAL PRODUCTION AND AVAILABILITY OF QUALITY FISH SEED IN ASIA-INCREASING BENEFITS TO THE RURAL POOR

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Shortages of large, predator-resistant seed remain a major constraint to the success of promoting rural aquaculture. Larger juveniles mean more success for food fish producers but strategies to ensure their consistent supply remain undeveloped. This problem persists despite the success and spread of private sector hatchery production of the major species cultured. Private sector seed producers tend to only produce small seed and be located within enterprise clusters distant from their dispersed rural customers. We present a framework explaining the current status and review potential strategies to improve the situation for all stakeholders, including the final consumers of food fish. The historical, technical and social contexts of fish seed production and distribution are considered. Both the location of production and the fish species determine potential approaches to produce quality juveniles close to their use and these are explored. The importance of fish seed supply strategies to the poor is discussed and the impacts of different approaches on them are assessed.

CONTRIBUTION OF SELF-RECRUITING SPECIES OF AQUATIC ANIMALS PRODUCED IN FARMER-MANAGED SYSTEMS TO URBAN FOOD SUPPLIES IN NORTHERN VIETNAM

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Aquaculture in rural areas located close to urban centres is often affected by market demand and input supply. A series of structured activities with groups of selected household representatives followed by a year-long longitudinal survey were conducted in communities within two districts of Red River Delta Northern Vietnam. The main objective of the research was to evaluate the importance of self recruiting species (SRS) in farmer-managed aquatic systems (FMAS) to the overall livelihoods of people. The two districts were selected based on their topography and their proximity to perennial water bodies; three villages in each district participated in the research. The study used participatory approaches to establish and understand the importance of various SRS to producers and other stakeholders.

Participatory product flow analysis conducted with focus groups was used to understand the flow of products from farmer-managed aquatic systems. Monthly monitoring over 12 months showed that most products were consumed by the household or sold in District-level markets or Hanoi; households in the upland communities studied tended to consume more. Evidence from a survey of 12 markets indicates that self recruiting species like freshwater prawns snakehead, tilapia, and snails were a significant component in urban District markets

UPTAKE OF TILAPIA GENETICS-BASED TECHNOLOGIES THROUGH PUBLIC-PRIVATE PARTNERSHIPS

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There are four strains of genetically-improved Nile tilapia (*Oreochromis niloticus*) in the Philippines which are being commercially distributed through public-private sector partnerships. Two modes of access are being practiced: open access; and through licensing, certification or accreditation. The establishment of various distribution pathways and corresponding mechanisms has resulted in increased availability of improved tilapia strains in the Philippines.

This study evaluated the effects and impact of public-private partnerships on access to and uptake of the products of tilapia genetics research. The research also looked at the dissemination procedures for the delivery of research products; product recipients or beneficiaries; access to and uptake of the technologies. The study has resulted in making recommendations for policy formulation.

Majority of the users of genetically improved tilapia are small landowners indicating that genetics-based technologies for tilapia tend to be scale-neutral. However, a high amount of capital is required in order to operate a tilapia farming business which may be beyond the reach of poor farmers. It is deduced that farmers using improved strains of tilapia, although generally not owning large tracts of land, are well-off because they have ready access to capital from their own sources thus marginalizing small farmers.

Results also show that technology transfer is not very evident, especially in areas which are not effectively served by government extension services. There is also concern about the perceived competition between the public and private sectors in the marketing and distribution of improved tilapia seed in certain areas. Hence, there is a need to identify specific functions of public and private sector in production and distribution of improved strains of tilapia seeds. It is expected that the proposed establishment and institutionalization of a Fish (Tilapia) Seed Certification System can address the growing concerns and anxieties over quality of seed and insuring integrity of improved tilapia.

IMPACT OF ENHANCED AQUACULTURE KNOWLEDGE ON THE SOCIO-ECONOMIC STATUS OF FISH FARMERS IN BANGLADESH

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Training in seasonal pond aquaculture management to poverty selected farmers in Northwest Bangladesh resulted in a 29% increase in fish production compared to a control group of untrained farmers. This increase was measured three years after intervention had ceased. The net income from fish production also increased by 305% compared with 37% for untrained. A reduction in production costs was a major contributing factor. The financial value of this rise amounted to an additional 25% on the annual average earnings of an agricultural labourer, which was the main occupation for 87% of both groups prior to intervention.

Despite increased financial assets there was no obvious improvement at this stage in socio economic status for the trained group over the untrained farmers. However, a more in-depth analysis of livelihoods issues after a longer interval would possibly provide better indicators of accrued benefit and present designers of future intervention activities with a clearer understanding of how advancement of the poor from technology transfer is realized.

SMALL-SCALE AQUACULTURE IN UPLAND AREAS OF YEN CHAU DISTRICT, SON LA PROVINCE / NORTHERN VIETNAM

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Within the framework of the special research program "sustainable land use and rural development in mountainous regions of southeast Asia" a survey on aquaculture practice was carried-out in Yen Chau district, son la province (Northern Vietnam). The region is classified as poor. Depending on the village, 30 – 95% of all farmers own ponds. About 80 farmers in a perimeter of 20 kilometres around Yen Chau town operating grow-out pond were interviewed on their farming activities. All belong to the ethnic minority of black Thai.

Typically, the Black Thai farmers live in villages in the valleys and cultivate maize, cassava and occasionally cotton as cash crops on the hillsides. Most farmers have smaller paddy plots in the valleys. Usually, the rice is only for home consumption. Often farmers possess one or more buffalos, some farmers own cattle and pigs additionally. Many also raise chicken and ducks. Fish ponds are mostly located between the paddy fields or next to the houses of farmers. The ponds (size between 200 and 5 000 m², most ponds below 1 000 m²) are characterized by a continuous water-flow. Usually, the farmers raise grass carp, mud carp, common carp and tilapia, in many cases also silver carp and silver barb (mean stocking density: 1 fish/m²). Around 30% of all stocked fish are grass carp. Fry and fingerlings are obtained from the local hatcheries. Tilapia and common carp are often not stocked due to natural reproduction.

The main feed inputs to the pond system are crop residues, such as banana, cassava, bamboo and maize leaves and rice bran. Different grasses and duck weed, normally collected from the paddy fields, are also fed. Almost all farmers manure their ponds with fresh manure (mainly from buffalo), but no animals are stocked in pens next to or above the pond.

The farmers usually harvest one or two years after stocking. The mean fish yield is around 240 g/m². A significant part (up to 100 kg/year) is consumed by the household itself or used as a gift item. The remaining part is sold to traders. In Yen Chau the prices for fish at the local market are generally considered as high (~ 1 US\$/kg grass carp). Prices for fish have further increased since the outbreak of bird flu in winter 2003/04. Farmers also collect snails, molluscs and shrimps from the pond. Fish farming is an important source of cash income and an essential animal protein supplement in the families' diets.

The fish yield of roughly 240 g/m² for a 2-year production cycle must be considered relatively low for a feed-based aquaculture system. Among the possible reasons for this are a) lack of training or extension services in the field of aquaculture, b) low quality of the stocking material, c) high mortality of grass carp due to diseases (84% farmers reported diseases of grass carp in former years) and d) negative impact of inorganic fertilizers and pesticides applied in paddy fields above the ponds.

Ongoing research aims at elucidating the nutrient flow patterns in this forage-based aquaculture system and at establishing nutrient flow models. Based on these models improved management schemes will be developed with the purpose of increasing productivity of the aquaculture system and income of the farmers.

LOCAL RESOURCE USERS GROUPS – AN APPROACH OF IMPROVING FARMER-MANAGED AQUATIC SYSTEMS

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Aquatic systems in flood-prone areas of South and Southeast Asia are characterized by linkages between household-managed and public water bodies. Sustained production of self-recruiting species (SRS) of aquatic animals appears dependent on their movement between the aquatic resources of neighbouring households and those accessed by the wider community. An improved understanding of the importance of SRS to rural livelihoods and the ecology of key species led to a framework for maintaining and improving their value to rural people based on the concept of local area resource management. Contiguous plots of land were identified in community meetings in 14 villages in lowland and more upland areas of southeast Cambodia, northeast Thailand and northern Vietnam. Current and improved management practices were identified and piloted by the local area groups and the outcomes monitored using participatory approaches over a single complete season. Most of the aquatic resources in the study areas were being managed at the household level. Local knowledge was particularly strong regarding factors affecting productivity of different types of water resource and life cycles of key species. Overall the common perception was that local group management resulted in increases in the availability of aquatic animals and a range of associated benefits. Important group management activities that was trialed and sustained to differ between lowland and relatively higher areas. For the higher areas, it was found that maintaining broodstock between seasons, either by retaining water in the refuge or trapping fish and stocking in a refuge were the most important activities. Retaining water in refuges was critical and complete pumping out of water from ponds uncommon. In contrast, the maintenance of broodstock is considered less important in lower areas and harvest of fish from trap ponds tends to occur over a prolonged period as opposed to a single occasion. Both location (high/low) and gender affected the relative importance of these activities. In general lowland farmers valued the benefits associated with group management more highly than farmers in higher areas. Farmers in both areas rated the benefits they gained as human capital highly i.e. increasing their knowledge and food for home consumption. Additionally men valued group management for the social benefits gained, whereas women tended to appreciate the physical benefits in terms of improving the rice field infrastructure and water control gains.

**ENHANCING FISHERIES DEVELOPMENT THROUGH FOCUSED SUPPORT TO STATE
FUNDED UNIVERSITIES IN BANGLADESH**

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From 1999 to 2004 support was supplied to five Government funded universities through fisheries related Faculty and Departments to enhance teaching delivery and improve ability to identify and conduct development focused research. This was achieved through a combination of competitive training and awards for course development and research. A total of 101 teachers secured awards to develop new or improve existing undergraduate courses and 52 teachers won 58 research awards. In addition training guided participants away from a generally insular campus focused attitude to research and dated teaching practice and content to development needs. These were identified through participatory approaches to the poorest in the fisheries sector and private sector agents. There has been a discernible improvement in teaching quality that has been noted by student and peer bodies. This shows every likelihood of continuing and providing teachers with the skills to compete successfully on the international stage for funding in research and being recognized in some disciplines as areas of excellence.

SEAWEED CULTURE AS A LIVELIHOOD OPTIONS FOR THE POOR COASTAL COMMUNITIES OF BANGLADESH

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Culture of seaweed was carried out for the first time in Bangladesh coastal water from February to May 2004. Two commercially important species; *Hypnea sp.* and *Caulerpa racemosa* at the St. Martin's Island were selected for the culture. Three different types of culture system (line, net and raft culture methods) were adopted. Seedlings were collected from natural sources. The growth rate was found to be 1.06 cm/day for *Hypnea sp.* and 1 cm/day for *Caulerpa racemosa*. The water temperature (27.5-31 °C), salinity (24-36 ‰), pH (7.3-7.5), dissolved oxygen (2.15-3.80 ml/l), alkalinity (108-120 ppm) and transparency (35.0-53.5 cm) were recorded in the coastal water of St. Martin's Island during the investigation period. The low salinity (24 ppt) decreased the growth rate of seaweed in the cultivated area of St. Martin's Island. Better growth was observed for *Hypnea sp.* and *Caulerpa racemosa* in net, raft and submerged tray method (net method). Lower growth of *Hypnea sp.* was observed in floating tray method probably due to the air temperature. The levels of protein, lipid, moisture and ash were estimated to be 10.75%, 5.17%, 76.26% and 7.37%, respectively, based on wet samples of *Caulerpa racemosa*. From the dry samples in *Hypnea sp.*, the levels of protein, lipid, moisture and ash were 13.35%, 3.59%, 21.76% and 17.78% respectively. The poor people living in the coastal areas will benefit from the introduction of seaweed culture as an alternative livelihood option. The construction material especially the bamboo is comparatively cheaper than PVC pipe and it is available in Bangladesh.

RAISING FISH IN DEEPWATER RICE AGRO-ECOSYSTEM: A NEOREALISTIC APPROACH FOR PROMOTION OF INDIAN RURAL AQUACULTURE

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India possesses about 2.5 million hectares of deepwater rice (DWR) area contributing 30% to world's total. Generally, the DWR agro-ecosystem is less favorable for rice (1.0-1.5t/ha/season) due to continuous water stress along with various other associated abiotic and biotic constraints. Being a seasonal wetland, the capture of wild fishery resources from the agro ecosystem is a common practice among the DWR farmers. The average of such wild catches hardly exceeds 100 Kg/ ha/seasons. During mid eighties, several culture trials were undertaken to investigate the production potential of deepwater rice and fish together for incorporating the system in rice based farming systems of the country. In this regard, a collaborative project of IRRI in India conducted multilocal culture experiments at 6-8 different DWR locations in West Bengal under treatment conditions of supplementary feed and no feed against the control of rice alone. The combinations of various carp fingerlings or carp fingerling & prawn juveniles were stocked @1no /m² with a stocking ratio of 50:35:15 respectively for bottom, column and surface feeders. In the field with feed treatment, Rice bran: Mustard oil cake was applied @ 2% of total body weight regularly and all the culture sites were maintained for about 120 days. DWR cultivation was done following normal agronomic practices of the locality of the experimental sites. The study revealed enormous potentiality (Table-1) to fit the stocked fishes into intricate aquatic food web for simultaneous production of fish along with rice. However, the utilization of DWR agro ecosystem for aquaculture development is still very scanty in India. Strengthening of the extension thrust for promotion of aquaculture in DWR would introduce a sustainable production technique leading to eco friendly and judicious utilization of these untapped aquatic resources of India.

Table1. Production potentials of rice & fish under deepwater rice fish culture experiments (multilocal) in West Bengal, India

Experimental condition	Crop	Production (Kg/ha)		
		1988 Kharif season (n =6)	1989 Kharif season (n= 8)	1990 Kharif season (n=6)
Control (Rice only)	R	920.0- 1800.0	3037.6- 4215.3	1804.1-3281.2
	F	-----	-----	-----
Rice + Fish (No feed)	R	1140.0- 2005.0	4067.0- 4873.5	1985.0-3614.0
	F	322.4 - 454.2	312.5 -1430.0	917.2- 1180.6
Rice + Fish (With feed)	R	1600.0-3230	4401.8-5735.0	1911.5 -3321.3
	F	508.2 – 674.4	574.0 - 825.1	684.2 -1909.1

F= Fish (carps/prawns), R= Deepwater rice (improved & traditional cultivars), n= Numbers of multilocal replicates

SOCIOECONOMIC PROFILE OF FISH FARMERS AND HOUSEHOLDS IN RELATED SECTORS: IMPLICATIONS FOR PRO-POOR DEVELOPMENT OF AQUACULTURE IN THE PHILIPPINES

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Socioeconomic profiles of fish farmers and households engaged in aquaculture in Taal and Sampaloc Lakes and in ponds in Laguna and Quezon provinces were compared. Fish species cultured and aquaculture technology adopted depended primarily on the type of resources available to the fish farmers. Fish culture in floating cages is practiced in lakes and was found more profitable than pond culture for the same fish species -- tilapia and milkfish. However, profitability differed between Sampaloc Lake and Taal Lake due mainly on the differences on stocking density and feeding practices. Catfish and prawn culture were basically pond-based. Catfish turned out to be the most profitable on a per m² basis but possesses its own unique problem - relatively poor market acceptance. A commonality observed in both cage and pond operations is that these are basically family-oriented undertakings. Men are involved in the production aspects and the women/wives, in marketing. Children assist in various aspects of the operations. Additional labor are provided by hired labor and "bayanihan" in the cases of Taal Lake and Kalayaan, Laguna, a form of mutual help offered by co-fish farmers, neighbors or friends on some one-time labor intensive activities such as stocking and harvesting. Knowledge and skills needed in cage and pond operations were acquired by most fish farmers through experience and training and do not require high educational attainment. While cage/pond operations serve as the major and in most cases the only source of livelihood, these remained peculiarly high-risk-low-return investments to many, and to some, simply a matter of no choice, as there is practically no alternative means of livelihood in their respective areas. Satisficing, rather than optimizing, has been the general norm in the study areas.

AQUACULTURE SUCCEEDS SUPERCYCLONE – SUSTAINABLE CARP CULTURE IN COASTAL AGRO-ECOSYSTEM OF ORISSA COAST (INDIA)

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 Management of Coastal Agro-ecosystem Affected by Supercyclone in Orissa
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The Super cyclone that hit the Orissa coast during 29-30th October, 1999 had completely ravaged the agro-ecosystem damaging crop, animal and fisheries activities. The water quality parameter studies indicated that super cyclone has completely destroyed the aquatic environment for pisciculture operations. NATP under World Bank support undertook a massive coastal agro-ecosystem rehabilitation programme. Different amelioration measures like removal of debris of biomass and silt, application of lime, bleaching powder, application of organic and inorganic fertilizers were undertaken periodically on a participatory mode in Panchayat and backyard ponds. The ponds were stocked with Indian major carps (IMC), Exotic Carps (EC), freshwater prawn and *Puntius gonionotus* seed material and standard composite fish culture practice was followed. A total of Panchayat ponds (area 13.2 Ac.) and 21 backyard ponds (area 13.5 Ac.) involving 449 families were evaluated under a participatory mode. An average fish yield of 2.7-3.0 t/ha in Panchayat ponds and 4.5-5.4 t/ha in backyard ponds were recorded, indicating the adaption of IMC, EC, freshwater prawn and *Puntius gonionotus* to the post-cyclonic coastal agro-ecosystem.

Table 1 On-farm trial on composite fish culture in cyclone affected community ponds and backyard ponds

Year	Species ratio	Stocking density (No./ ha)	Manurin g schedul e	Dose (kg/ ha)	No. of Install-ments	Feeding schedul e	Yield (kg/ ha)	B:C ratio
2001 - 2002	C.R.M.	More than 10,000	Only cowdung	200	Every month	RB : GOC 50:50	800	0.6
2002 - 2003	C.R.M. GC, SC, CC, Puntius, Prawn	6000 (10-50g size)	Lime Urea SSP Cowdung + Poultry litter	400 500 600 5000	2 2 2 15 days	RB+GNO C MM + Vitamins @ 2% body biomass	3500	1.9

C - Catla, R - Rohu, M - Mrigal, SC - Silver carp, GC - Grass carp, CC - Common carp;
 RB - Rice bran, GOC - Groundnut oil cake, MM - Mineral mixture

***SS Production in Aquatic
Peri-urban Systems in
Southeast Asia***



**THE CURRENT STATUS OF PERI-URBAN AQUATIC FOOD PRODUCTION SYSTEMS IN
SE ASIA: AN OVERVIEW OF FOUR CITIES, BANGKOK, PHNOM PENH, HO CHI MINH
CITY AND HANOI**

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The cultivation of fish and aquatic plants is widespread throughout many cities in SE Asia and is intrinsically linked with the livelihoods of a significant number of the lower income households who live there. As such the production systems involved are often less intensive requiring low and recycled inputs, and often utilising the city's domestic waste water as a source of nutrients and fertiliser for increasing production.

This paper gives an overview of the importance and impact of peri-urban aquatic production systems in the four, afore-mentioned SE Asian cities drawing on the first year's research and findings from the Papussa (Peri-urban Aquatic Production systems in SE Asia) project. Production, livelihood, markets and institutional aspects affecting a wide range of the stakeholders involved are discussed within the context of increasing urban migration, future sustainability and development in the sub- region, with comparisons highlighting similarities and differences between the four cities developed.

**URBAN DEVELOPMENT, LAND POLICY AND THE FUTURE OF PERI-URBAN AQUATIC
FOOD PRODUCTION SYSTEMS IN SOUTHEAST ASIA**

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Peri-urban aquatic food production systems are profoundly shaped by the dynamic development context in which they arise, develop and, often, decline and disappear. The urban fringe is a zone where environmental, social and economic systems are influenced by urbanization and industrialization and the needs and demands of different land uses and users. In some cases livelihoods and more widely development activities interlock in creative and mutually supportive ways; in other instances the points of contact are destructive and undermining. Furthermore, property rights systems and the institutional foundations in such transitional zones are often either in a state of flux or poorly developed. The paper will present a preliminary exploration of the history, development, nature, role and functioning of aquatic food production systems in four Southeast Asian cities (Hanoi, Ho Chi Minh City, Phnom Penh and Bangkok) with a view to identifying the key drivers of change and whether there is a characteristic 'transition'. The paper will also acknowledge, however, the critical role of national and local contexts in directing such transitions.

AN OVERVIEW OF THE CURRENT STATUS OF PERI-URBAN AQUATIC FOOD PRODUCTION SYSTEMS IN PHNOM PENH

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Similar to a number of other cities in Southeast Asia such as Hanoi, Ho Chi Minh City and Bangkok, peri-urban areas of Phnom Penh are very important sites for producing and supplying food produced from agriculture. Aquatic areas around peri-urban wetlands are one of the most important locations for cultivating aquatic plants and fish for the city and other areas of Cambodia as they are fertilized by sewage/waste water discharged from the city. The value and nature of these production systems are intrinsically linked with the livelihoods of many poor people living in and around the city. A study and appraisal of the value and impact of these systems is most important in order to understand their sustainability within the overall future development of the city.

The relative dependence of people livelihoods on these systems and a broad assessment of the importance of rural-urban linkages, relationships were conducted, including the motivation and legal frameworks affecting current participation in peri-urban food production systems. This research involved Participatory Community Appraisal, a Markets study and an overall Institutional analysis with various participants from different spectra of stakeholders being informed and asked to validate the results of the first year's research in a State of the System (SOS) stakeholders' workshop.

Four representative communities in and around Phnom Penh were identified and selected for this overview. Among these communities, two were involved in fish farming and two in aquatic plants farming (mostly morning glory cultivation).

It was found out that in amongst the two fish farming communities, the main problems and needs households were facing were slightly different from people whose livelihoods were based on aquatic plant farming. People whose livelihoods were based on growing aquatic plants in city wetlands wanted improvements in their basic living infrastructure whilst people/farmers growing fish in peri-urban area were more concerned about lower income, security of their farmed animals, wild fish resource depletion, and human health. Increasingly expanding industries and human settlement around the city's wastewater wetland is reducing the availability of land for farming, whilst lower incomes, lack of capital, negative effect on morning glory and human health; and reduction of wild fish were also major problems encountered.

It was found mainly in the market study that the main concerns were small profits, price fluctuation of their produce, and health issues. It was suggested that the government should improve new markets and export sectors, tax and also provide both business security and more appropriate marketing facilities.

It was mainly concluded that peri-urban food production systems and their value are very important and valuable for people's livelihoods. Modification of these systems can both negatively and positively affect their future

**STATE OF PERI-URBAN AQUACULTURE SYSTEMS, MARKETING CHANNELS OF
AQUATIC PRODUCTS AND INSTITUTIONAL STRUCTURE FOR AQUACULTURE IN HO
CHI MINH CITY**

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Participatory Community Appraisal (PCA), a modified version of the PRA technique was applied to define the state of peri-urban aquaculture systems in HCMC. The study was done at 4 representative peri-urban communities located in Binh Chanh, Hoc Mon and Cu Chi districts. Aquaculture systems, farmers' activities, resources use, and problems were described by farmers themselves that reflect true farmers' livelihoods as well as the roles of aquaculture within their communities. Wastewater dependence of different systems in different places can have connection to farmers' health and technical difficulties. Geographical location of a community decides its risk and uncertainties caused by urbanization. Marketing problems are resulted from poor access to market.

A survey was carried out with 67 markets in 22 districts of HCMC. Different types of markets and market actors were involved in the survey. Gender is significant in the roles played by different market actors. Whilst most collectors are men, wholesale and retail activities are mainly done by women. Marketing channels of fish and aquatic plants are complicated in which products are shifted through many intermediate trading steps before coming to consumers. Different market actors may sell their products to one or many kinds of buyers. A large proportion of fish is sold live in markets. All aquatic plants are sold fresh in markets and mostly supplied from nearby peri-urban areas. Fish and aquatic plants are transported to markets by different means showing the transportation requirements are distinct. Seasonal fluctuations of prices for both fish and aquatic plants were found but the fish price is less influenced by seasonal factors. Captured fish are preferred by most consumers. Administrative factors and labor requirements are diverse among market actors.

Many institutions are involved and play important roles in the development of peri-urban aquaculture. Roles and functions of these institutions were investigated by open discussions with resource persons at respective institutions. Although the administrative structure is well organized, the related institutions are not in close contact or connected when they are carrying out planning. The Government is not concerned enough in the development of aquaculture within HCMC. Industrial sector and service are assigned to be increased in the development of the City's economy. This relative governmental neglect of aquaculture in the urban environment may result in the future in the reduction of aquacultural areas within the city.

THE STATUS OF PERI-URBAN AQUATIC PRODUCTION SYSTEMS IN HANOI

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The dynamic and rapid growth of Hanoi City (Vietnam) has expanding urban areas and led to increasing opportunities for production and trading of aquatic food. Changing land use and water in and around city also affects the communities which are producing aquatic food. Recently, Participatory Community Appraisal was conducted in 4 representative communities around Hanoi, and marketing and institutional aspects were surveyed, analyzed, presented to different aquatic production stakeholders for their feedback. This paper presents a brief overview of recent status of peri-urban aquatic production systems in Hanoi, including an understanding of the communities, markets and the institutions involved.

Wastewater is commonly used for aquaculture and aquatic vegetables production in and around Hanoi, but levels of wastewater used are different from commune to commune, high levels of wastewater with production systems in communities that are close to the main wastewater canal. Whereas communities further away from sewage sources, very low levels and not using wastewater are commonly practiced in aquatic production. Diversification, integration and intensification of aquatic productions i.e. fish culture, rice-fish; vegetables are common practices in most of aquatic production systems in Hanoi peri-urban areas. Impacts of urbanisation and changes in land use policy, roles of women and men, responsibilities of different aquatic production stakeholders in the recent development of Hanoi peri-urban aquatic production are described. Existing constraints and options for future development of Hanoi aquatic production are also discussed and recommended.

STATE OF PERI-URBAN AQUATIC FOOD PRODUCTION SYSTEMS IN BANGKOK

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This is a preliminary overview of the current peri-urban aquatic food production systems in Bangkok describing the livelihood of farmers involved in peri-urban aquatic food production systems, resource use and their existing problems. During the first year of the project, Market Analysis (MA), Institutional Analysis (IA) and Participatory Community Appraisal (PCA) have been conducted via primary data collections (field survey, interview and workshop seminar) and secondary data collections (published documents and information).

Markets Analysis was carried out through 665 market-related individual-stakeholder survey questionnaires in 16 market places in and around Bangkok in relation to their demographic information, market channels, sources of produce (aquatic plants and fish), transportation, marketing costs, factors affecting prices, market administration, requirement of additional labor, and future trends of business.

Institutional Analysis based on reviewing of published documents and information was conducted. The summary of National Economic and Social Development plans in relation to economic development and city planning has been summarized. The information on central-level institutions involved in peri-urban aquatic production was also analyzed and included.

Participatory Community Appraisal was arranged through workshop seminars in 4 communities representing Bangkok peri-urban areas within Pathumthani, Nontaburi and Samut Prakarn provinces. Information in relation to wealth ranking, community mapping, historical timeline, seasonal calendars, activity matrix, food consumption, resource mapping and problem ranking were monitored.

Within peri-urban Bangkok the major aquatic products cultivated include aquatic plants such as morning glory intensively farmed in fertilized converted ricefields in Nong Prao Ngai sub-district, Sai Noi district, Nontaburi province, water mimosa farming in irrigation canals in Suan Prik Thai sub-district, Muang district, intensive hybrid catfish pond culture found in Lumsai sub-district, Lumlukka district, Pathumthani province, and mixed tilapia and carp pond culture in Srisajorakae Noi sub-district, Bang Sao Thong minor-district, Samutprakarn province. These systems and communities are located within the extended Bangkok Metropolitan Region (BMR) with risk and uncertainty to them caused by rapid growth of urbanization, whilst conversely the benefits associated with the proximity of a large markets and potential consumer base.

**EVALUATING PARTICIPATORY ACTION PLAN DEVELOPMENT IN FORMULATING
SUSTAINABLE MANAGEMENT STRATEGIES FOR PERI-URBAN AREAS, WITH
EMPHASIS ON THE EAST KOLKATA WETLANDS**

Dr. Nitai Kundu, Mousumi Pal, Samarpita Ray Chaudhuri, Stuart Bunting

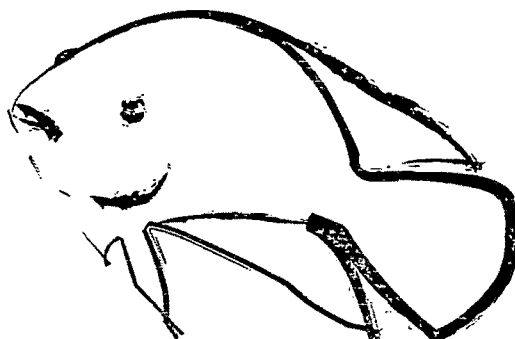
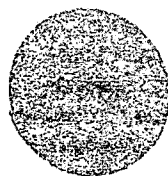
Past research has identified several technical constraints to sustained or enhanced production from peri-urban farming around Kolkata, in particular problems in accessing reliable wastewater and solid organic waste inputs, dependence on externally sourced high cost inputs, recurring disease problems and limited access to appropriate credit arrangements. However, it would be dangerous to assume that increased productivity or profitability for peri-urban farming would contribute directly to enhanced livelihoods for poor people. Research findings also demonstrated that poor people were often keen primarily to enhance their cash income and that with adequate support this might be obtained from urban-based activities. Whilst enhanced agricultural production would contribute to sustaining poor livelihoods, to move out of poverty many people in peri-urban Kolkata require access to alternative livelihood options, and for young people, education, training and knowledge of transaction costs in moving to different livelihood strategies are required to access such opportunities. Furthermore, rising expectations combined with uncertainty regarding prospects for peri-urban farming in the face of urban growth are promoting diversification of household livelihood strategies.

Having assessed the situation it was clear that decision-making and action planning to address problems faced by poor people should involve collaboration between key stakeholder groups. However, the divergent interests and agendas of these groups, especially the poor and urban and rural government agencies, suggested further research, in particular process monitoring, was required to evaluate participatory action plan development. This approach has reportedly been effective in rural situations but remains to be tested in a complex social and administrative peri-urban context.

The current project aims to allow everyone involved in sustaining the wetlands a voice in its future management through action planning, providing an opportunity for the Government, landowners, labourers and others to meet together and agree upon a set of actions that will benefit all concerned. This will not just be a list of hopes, but will set out clear steps by which these ideas can be implemented. Findings from previous research have resulted in the action planning process focusing on access to wastewater, a key constraint affecting the majority of poor people whose livelihoods depend either directly or indirectly on horticulture, paddy farming or aquaculture in peri-urban Kolkata. In the future it is hoped knowledge of action planning in peri-urban areas will be useful in addressing broader issues such as livelihoods diversification and service provision.

New knowledge on implementing action planning with poor people in peri-urban areas and rural and urban agencies is being assimilated, and it is intended to communicate this in formats appropriate for the various stakeholder groups it should influence, locally, nationally and internationally. If the project is successful it is hoped that the action planning tools developed could be used to assist in the planned management of other peri-urban areas and wetlands in West Bengal, India and globally. Furthermore, potential donors are being made aware of the action planning process and will be asked to contribute to ensuring that the wishes of all those with an interest in the management of the wetlands can be met.

SS Aquatic Ecosystem Health Management



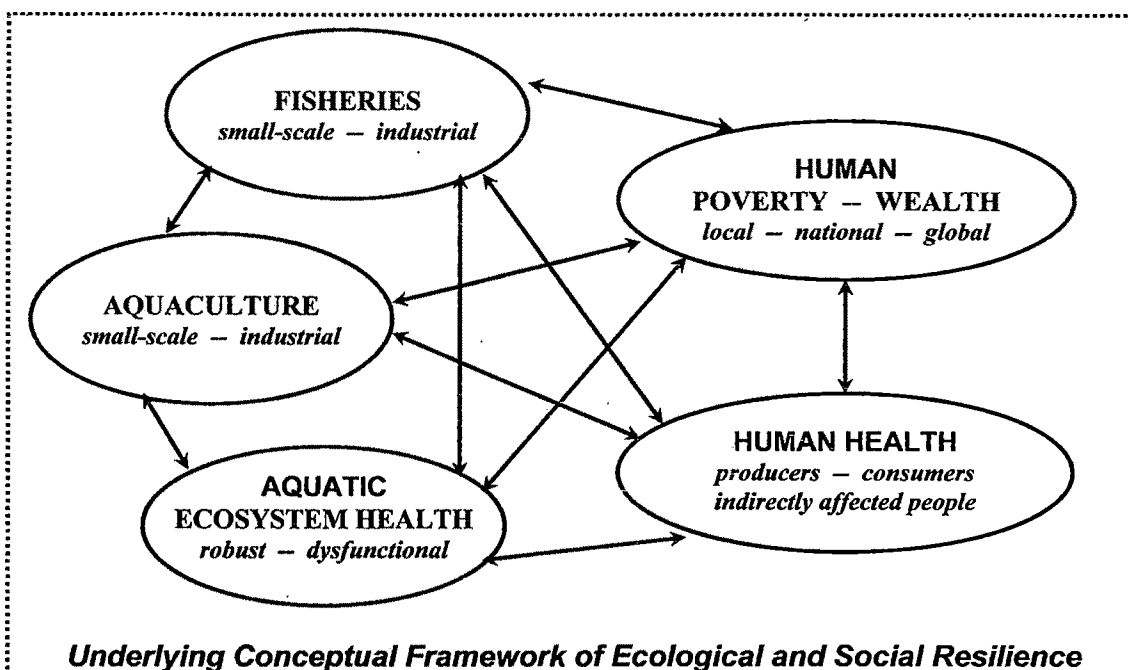
LINKAGES AND INTERACTIONS BETWEEN FISHERIES, AQUACULTURE, AQUATIC ECOSYSTEM HEALTH, HUMAN POVERTY / WEALTH AND HUMAN HEALTH

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This paper reviews the linkages between fisheries, aquaculture, aquatic ecosystem health, human poverty/wealth and human health. It explores both positive and negative processes. We analyse key interactions with examples from research in Norway, Tanzania, Bangladesh, Malaysia and Vietnam. We conceptualise these within a framework of ecological and social resilience in quest of sustainable aquatic ecosystem management and human well-being.



Aquatic ecosystems often function well in the context of small-scale traditional fisheries and aquaculture systems, whereas some industrial cases involving large-scale trawling or the monocultures of carnivorous species cause ecological dysfunction, especially when poorly managed. Overfishing and use of destructive gears seriously damage fisheries sustainability and jeopardise aquatic ecosystem health by disrupting natural resources and ecological service functionality. Pollution from aquacultural wastes and the persistent use of toxic chemicals, medicines and antibiotics against diseases endanger ecosystem health and may exacerbate hazards of drug-resistant pathogens and antibiotic-resistant bacteria.

Fish captured and cultured provide nutrition, improved health and raised incomes for people, but the benefits are often uneven - locally, nationally and globally. Unwise and unsustainable culture practices may spread diseases and pathogens to humans and confer biomagnified concentrations of toxins thereby constituting a threat to hygiene and human health safety. Feeds for carnivorous species of fish and crustaceans demand huge quantities of fish oil and protein: it is the epitome of non-sustainability when one uses several tons of captured fish to produce a single ton of farmed fish/shrimp where fish from the oceans is increasingly scarce. Production of luxury export items may be profitable for individual commercial enterprises in the short-term, but increasingly threatens food security and health of poor people in low-income countries by rendering even small fish unaffordable. Unfair trade in fish products reinforces international inequalities. Globalization in these forms exacerbates poverty.

RECENT ENVIRONMENT DAMAGE AND WATER MANAGEMENT SYSTEM OF SHRIMP CULTURE ON THE ANDAMAN SEA COAST, SOUTHERN THAILAND

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Shrimp culture has become the highest revenue-earning sector in coastal aquaculture. In Thailand, production has shifted from extensive and semi-intensive to intensive culture since artificial propagation techniques became economically practical. After serious losses that occurred in the upper gulf and on the east coast of Thailand, shrimp farmers moved to first to the southeastern coast and then across the peninsula to the Andaman Sea coast. Shrimp farmers have tended to shift their cultivation from an open water management system to a closed system, even if the profit rates decrease. The reason for this shift is to avoid mass mortality caused by poor external water quality and by introduction of infectious disease agents or their carriers. Although regulation of shrimp farming has been introduced, there has been a poor response to the directive for registration of all farms greater than 8 ha and for construction of effluent settlement ponds. Small shrimp farms with water areas lower than 8 ha represent more than 89% of all shrimp farms on the coast of the Andaman Sea. Since they are not covered by regulations, the majority continue to discharge untreated effluent. This is one of the main reasons why shrimp farming on the Andaman Sea coast may not be sustainable. Many recommendations have been made to improve shrimp farming practice. However, implementation requires that the government promote environmental recovery where deterioration has occurred through farmer cooperation in management practices including discharge of effluent into receiving canals. It is likely to be difficult to reach agreement on coordination terms.

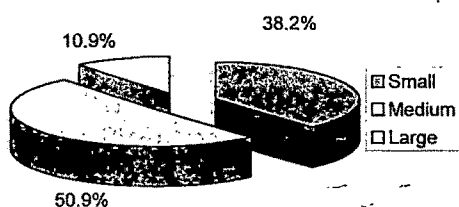


Figure 1 Distribution of farm sizes on the Andaman Sea coast.

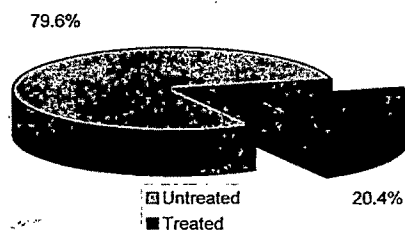


Figure 2 Management of effluent from shrimp farms.

FECAL CONTAMINATION IN OYSTER *Crassostrea iredalei* CULTURED IN SETIU LAGOON, TERENGGANU, SOUTH CHINA SEA

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The levels of *Escherichia coli* and fecal coliform bacteria in water, sediment, and oyster (*Crassostrea iredalei*) cultured in the Setiu Lagoon, Terengganu, South China Sea were studied between August and December 2003. Twelve sampling stations were established in this study and they were visited three times during the southwest monsoon, inter-monsoon and northeast monsoon seasons. The mean values of *E. coli* in water, sediment and oyster were 5.19 MPN/100 ml, 0.29 MPN/g and 0.80 MPN/g respectively. While for the mean values of fecal coliform bacteria counts, the values were 41.40 MPN/100 ml, 0.29 MPN/g and 2.85 MPN/g respectively. The mean value of *E. coli*, MPN/g oyster decreased from 0.92 in August, to 0.69 in October, and then to <0.4 in December. The heavy monsoon rains have a strong influence on the bioaccumulation of *E. coli* in oyster due to the dilution rate of the water. The fecal coliform counts in water exceeded the water quality criteria of 14 MPN/100ml set by DOE, Malaysia. This reveals that the Setiu Lagoon is contaminated with domestic sewage. The fecal coliform and *E. coli* contamination in oyster cultured in the Setiu Lagoon are still within the safety level for human consumption set by United Kingdom for the Microbiological criteria for shellfish. A proper measurement on the control of sewage pollution in this lagoon is urgently needed for protecting the environment as well as to ensure the hygienic sea food production.

USE OF MOLECULAR MARKERS TO DETERMINE THE SPECIATION OF *Perkinsus* sp. COLLECTED FROM MANILA CLAMS *Ruditapes philippinarum* IN KOREA

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Perkinsus is the causative pathogen responsible for mass mortality of the Manila clam, *Ruditapes philippinarum*. *Perkinsus* sp. isolated from Manila clams collected in Korean waters was assayed by the polymerase chain reaction (PCR) to determine its phylogenetic affinity with other congeneric species. Regions of rRNA of *Perkinsus* sp. isolated from clam haemolymph were cloned and sequenced. Sequences of a non-transcribed spacer (NTS) and internal transcribed spacers (ITS 1, 2) flanking 5.8S rRNA were compared to available sequences of *Perkinsus* spp. The NTS sequence of Korean *Perkinsus* was 99.91 - 100% similar to that of *P. atlanticus* and 98.06 - 98.15 and 73.05 - 73.14% similar to those of *P. olseni* and *P. marinus*, respectively. The ITS 1, 5.8S rRNA and ITS 2 sequences of Korean *Perkinsus* showed 100% similarity to *P. atlanticus* and *Perkinsus* sp. reported from Japan. The ITS-5.8S rRNA sequences of Korean *Perkinsus* were 99.86 and 93.73% similar to those of *P. olseni* and *P. marinus*, respectively. The sporulation pattern and morphology of the Korean *Perkinsus* were very similar to those of *P. atlanticus*. Our data strongly suggest that the *Perkinsus* sp. isolated from clams in Korean waters is the same species as *P. atlanticus*, which is distributed in European waters.

PROBIOTICS: A BREAKTHROUGH IN AQUATIC HEALTH MANAGEMENT

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Globally, aquaculture is expanding rapidly. The FAO estimates that by 2020, half of the world's sea food demand will be met by aquaculture. In India, aquaculture industry has also been growing tremendously in the last two decades. India has achieved fourth position in fish production among all fish producing nations of the world. The contribution of aquaculture to the country's shrimp export yielded Rs. 4,235 crore during last year, and as much as 89.57 per cent of it was contributed by aquaculture.

In India, the average survival rate of shrimp larvae is reported to be around 25-30%, while in Thailand, the average survival is 50-60%. The reasons may be food, water, nutrients and /or diseases. Disease outbreaks are being increasingly recognized as a major constraint in aquaculture production and trade, affecting the economic development of the sector. So far, conventional approaches, such as use of disinfectants and antibiotics. Have limited success in the prevention and cure of aquatic diseases. Moreover, antibiotic use has various ecological considerations such as development of antibiotic resistant microbial strains, transmission of resistant strains, threat of residual toxicity leading to bioaccumulation/biomagnifications and persistence of low biodegradable substances in the environment for longer time. Along with disease outbreaks, aquaculture is also facing other challenges such as water pollution, water quality management etc. All these situations present considerable scope for biotechnological and other technology interventions.

The convention on biological diversity defines biotechnology as, "any technological application, that uses biological systems, living organisms or derivatives there of, to make or modify products or process for specific use". In this regard, the term "Probiotics" comes in, which may be termed as 'microbial biotechnology'. Probiotics are the cultured products or live microbial feed supplements, which affects the host by improving intestinal balance and health of the host. The first probiotic discovered long time ago was *Lactobacillus* sp., the lactic acid producing bacteria. They were thought to prevent colonization of the gut by other disease causing bacteria, a process known as 'competitive exclusion'. Presently, the range of probiotics extend well beyond the *Lactobacillus* to include wide range of gram positive (*Bacillus*, *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Micrococcus*, *Streptococcus* and *Weissella*), gram negative bacteria (*eromonas*, *Alteromonas*, *Photorhodobacterium*, *Pseudomonas* and *Vibrio*), microalgae (*Tetrasemis*), Yeast (*Debaryomyces*, *Phaffia* and *Saccharomyces*) etc. Probiotics can directly be applied with the feed and binder (egg and cod liver oil) or indirectly to water for the improvement of water quality by affecting fertilization and also by decomposition of organic matter (gram negative bacteria are more efficient than gram positive bacteria). However, the mode of action of the probiotics is rarely investigated, but the possibilities include competitive exclusion, alteration of microbial metabolism and/or by stimulation of host immunity. The present paper deals with the role of different probiotics in aquaculture with special reference to Indian scenario.

SURVEY ON BACTERIAL FLORA AND HEAVY METAL CONCENTRATIONS IN WILD OYSTER *Crassostrea iredalei* FROM SETIU WETLAND, MALAYSIA

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Slipper oyster *Crassostrea iredalei* is a species of good demand in Malaysia for its sweet flavor and white colored flesh. The filter-feeding nature predisposes oysters to accumulation of pathogenic bacteria and heavy metals in waters impacted by sewage pollutions, and may thus render the oysters unfit for human consumption. A survey was undertaken to study the bacterial flora and heavy metals concentrations in wild slipper oysters from Gong Batu, the Setiu wetland in Terengganu. Bacteria were isolated using selective (TCBS, GSP, DHL, MacConkey, Levine EMB, Baird-Parker and Broth) and non-selective (TSA) agars, and identified using conventional methods in combination with BBL Crystal™ Kit. Heavy metals (zinc, cuprum, cadmium and plumbum) concentrations were determined using atomic absorption spectrophotometry.

The oysters were found to harbor predominantly *Shewanella putrefaciens*, followed by *Aeromonas hydrophila*, *Enterobacter cloacae*, *Vibrio parahaemolyticus* and *Chromobacterium violaceum*. The oysters were found to contain high concentrations of zinc, cuprum and cadmium relative to the maximum concentrations allowed in fish and fish products under Malaysia Food Act 1983. The plumbum concentration was however within the safety limit (Table1).

Table 1 Heavy metals concentrations detected in wild slipper oyster *Crassostrea iredalei* from Setiu wetland, in comparison with the maximum concentrations allowed in fish and fish products under Malaysia Food Act 1983.

Element	Present study (ppm)	Maximum concentration allowed (ppm)
Zinc (Zn)	785.68 ± 285.88	100
Cuprum (Cu)	38.9 ± 13.2	30
Cadmium (Cd)	1.60 ± 0.28	1
Plumbum (Pb)	0.17 ± 0.15	2

CATCH RATES AS AN INDICATOR OF ECOSYSTEM HEALTH AND EXPLOITATION STATUS IN THE SHRIMP FISHERY IN SOUTH VIETNAM

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Based on an analysis of enumerator catch and effort data covering the period 1996-2002, time series of catch rate indicators from two major landing places in South Vietnam: Ganh Hao in Bac Lieu province (facing the South China Sea) and Song Doc in Ca Mau province (facing the Gulf of Thailand) are presented.

The indicators include catch rates for total catch, total shrimp catch, five major shrimp catch groups and 15 commercial shrimp groups estimated as monthly averages by fleet.

The analysis indicated that despite a reduction in catch rates, shrimp is still the most important resource for the trawl fleets in South Vietnam. Furthermore, it was found that there are significant differences between the two areas with regard to both total shrimp and catch group catch rates. However, in both provinces the cat prawn contribution fluctuated in counter phase with the low value shrimp contributions.

The enumerator data indicated that there might be seasonality in the shrimp catch rates that could reflect seasonality in shrimp recruitment making this resource potentially suitable for a fishery management system based on closed seasons.

Based on the experiences from the present analysis recommendations are given with regard to adjustments of the enumerator data collection programme to fulfill the requirement of a dynamic fisheries management system.

IMPACT OF WHITE SPOT DISEASE ON SHRIMP CULTURE INDUSTRY IN SRI LANKA

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Sri Lankan shrimp culture industry was first affected by white spot disease in mid 1996. Approximately 948 farms covering an area of nearly 2500 ha were operational at this time. Over 80% of the farms became non operational within 2 months of the disease outbreak. Hereafter, less than 90% farms were operational at any given time of the year. The disease however continues to be a serious problem.

The following measures were taken to reduce the risk of White spot disease, 1) use sedimentation and treatment ponds for incoming water supply, 2) use chlorine to treat the water, 3) practise semiclosed or recirculation systems, 4) use probiotics to treat pond water, 5) use of other biological treatments to purify incoming water, 6) use pesticides and other chemicals such as chlorine and disinfectants in shrimp ponds to kill diseased shrimp and 7) screen larvae for WSV prior to stocking. However, none of these were 100% successful in controlling the disease although some treatments helped to reduce the risk by about 40%.

Prior to the white spot disease many symptoms related to water and sediment quality were reported to cause mass mortality mainly due to low oxygen levels in the culture ponds or due to toxic effects of accumulated waste products. Heavy bacterial and protozoan infections also resulted in mass mortalities. Currently, farmers do not pay attention to any other shrimp disease or symptoms and any mortality observed are always attributed to WSV which is followed by quick harvesting. Present investigation, however, showed that many harvests are as a result of other common disease symptoms which are falsely recorded as WSV.

White spot disease has also caused over 30% farms to be abandoned and these have created other social problems including creation of mosquito breeding habitats and spread of diseases such as filariasis, dengue fever, encephalitis and malaria. It has also affected the socio economic status of the area with 28% inhabitants of the area being rendered jobless due to closing of farms during most parts of the year.

The other impacts of white spot disease are, 1) poor income in shrimp culture areas 2) excess use of chemicals and pesticides, 3) pollution or water quality changes in the water supply caused by use of chemical and nonchemical water treatment methods in farms. All this has caused continuing problems in producing good quality shrimp. The loss of confidence in the industry on the part of donor agencies has caused severe financial problems reducing the possibility of reviving the industry.

Currently, at any time of the year less than 40% farms are in operation due to fear of White spot disease. Therefore there is an urgent need to document the actual problems in the shrimp industry and guide the farmers, specially the small scale farmers who do not have access to new technologies, to overcome these problems. This is especially because the farmers do not have access to internet or availability of published material on new technologies.

CHALLENGES AND THREATS TO THE ECOSYSTEM HEALTH AND SUSTAINABLE DEVELOPMENT: LESSONS FROM THE NORTH AMERICAN GREAT LAKES

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The North American Great Lakes have suffered from eutrophication during the past. The deteriorating state resulted in the bi-national application of phosphorus abatement strategy by Canada and the United States under the Great Lakes Water Quality Agreement (GLWQA), which brought about a substantial improvement in water quality. During this recovery period, numerous invasions by exotic species posed a new threat to the Great Lakes food web. While the presence of certain exotic species can be traced back to Europe, the appearance of zebra mussels in 1988 piqued scientific and public interest. The combination of phosphorus abatement and invasions by zebra and quagga mussels (*Dreissena polymorpha*; *D. rostriformis bugensis*) resulted in a drastic reduction of phytoplankton and increasing water clarity in the lower Great Lakes. The dreissenid invasions were followed establishment of by other exotic species including the round goby (*Neogobius melanostomus*) and fishhook waterflea (*Cercopagis pengoi*). Assessment of pre and post invasion periods demonstrated drastic biotic alterations at the base of the food web leading to the decline of healthy Great Lakes fisheries. Lake wide monitoring programs in the Great Lakes were initiated in the early seventies for assessing the impact of eutrophication and contaminants. These monitoring programs have played a key role in the conservation of these large bodies of water. Currently expanded environmental monitoring programs are extremely important for assessing the invasion of exotic species, status of species at risk and sustainable development strategies. Examples from the Great Lakes will be discussed to emphasize the role of integrated and holistic monitoring for timely assessment of food web alterations and fisheries with the help of powerful ecosystem modeling.

EFFECT OF A RECLAMATION PROJECT ON NUTRIENT DISTRIBUTION IN ARIAKE SEA, JAPAN

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Under the Isahaya Bay reclamation project in Japan, the sea dike closed about one third of Isahaya Bay in 1997. The study examined nutrient concentrations at 16 to 20 sampling stations in the regulating reservoir (almost freshwater pond at present within the dike) and 36 to 92-stations in Ariake Sea outside of the sea dike in various seasons since 1977. The data indicated that mean concentrations of nutrient in the reservoir increased after the closing of Isahaya Bay. For example, nitrate plus nitrite-nitrogen increased from $0.6 \pm 0.3 \mu\text{M}$ in 1997 to $200 \pm 48 \mu\text{M}$ in 2000. The high nutrient water in the reservoir seems to be brought by the serious damage of the tidal flat ecosystem, which had functioned to remove the nutrient until the dike construction. The nutrient rich water in reservoir was intermittently drained into Ariake Sea through the two floodgates. It was observed that high nutrient and low salinity water mass often appeared in the surface water in Ariake Sea. This water mass seemed to originate from discharge of nutrient rich and small density fresh water of the regulating reservoir, and to spread into the Ariake Sea due to physical process such as mixing. It is concluded that the dike construction in Isahaya Bay effected a change of nutrient distribution in the Ariake Sea.

ENVIRONMENTAL CAPACITY AND MANAGEMENT OF COASTAL SHRIMP CULTURE IN BANGLADESH

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It is commonly assumed that expansion of coastal aquaculture in Bangladesh poses risks in exceeding environmental capacity. TROPECA, a DFID-funded international research project involving Nautilus Consultants and Stirling University (UK), Fisheries and Marine Resource Technology Discipline, Khulna University and University of Dhaka (Bangladesh) and RIA1, The University of Fisheries and the University of Agriculture and Forestry (Vietnam) investigates this issue with case studies in Bangladesh and Vietnam. From 2002 the team in Khulna has been working closely with an NGO (UTTARAN) studying an aquatic system comprising a river (Semi diurnal tidal system), a canal and 25 shrimp ponds.

Shrimp culture practices were mostly extensive to improved extensive system. Shrimp are being produced without proper pond preparation, fry nursing, stocking or feed management due to a lack of technical knowledge and also concern about the risks associated with higher levels of investment. Consequently production rates are low, averaging only 207 Kg/ha in the study area. Very high mortality of shrimp was reported in some of the farms using shallow ponds immediately after heavy rainfall. This may be have been due to the reduction of water pH and/or fluctuation of water temperature and salinity. The team encouraged farmers to report conditions leading to a variety of potential indicators being tested (reddish color, oily layer and salt bubble of water, as well as bad odor of soil) to indicate poor water and soil quality prior to shrimp mortality events.

About 45% of total N content in feed was converted by shrimp, the remainder reaches the ponds as uneaten feed, feces or excreted matter. About 27 % of total N inputs were transported out of the ponds by periodic water exchange. Nitrogen load in the ponds is estimated as 37.1Kg N per ton of shrimp produced per year, which is very low, compared with the intensive culture system seen in other countries. Year round water quality observations indicated that there is limited organic pollution in the aquatic systems with water quality in ponds reflecting changes in the surrounding environment. It is possible that indicators of poor pond water quality correspond to specific periods of intensive rice production in the surrounding area. This is to be further investigated under the project.

It may be concluded that the aquatic environment is able to accommodate the present level of aquaculture practice. Indeed it may be that shrimp culture is sporadically being adversely affected by the intensive rice culture. The assimilative power of aquatic environment is in danger of being degraded should the intensification of shrimp culture areas occur. Given the poor yields investment in more intensive culture has been slow to materialize. It may therefore be argued that the impact of rice culture is therefore constraining the development of shrimp culture in the area. Such cumulative impacts must be considered in planning aquaculture and other development if environmental capacity is not to be exceeded.

STEPWISE REGRESSION ANALYSIS FOR DISSOLVED OXYGEN WITH OTHER WATER QUALITY PARAMETERS DURING HIGH TIDE IN A TROPICAL ESTUARY

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Dissolved oxygen is one of the most important factors for the survival of aquatic animals and it is a unique indicator of the health of aquatic ecosystems. Since dissolved oxygen level of a water body is always subject to changes due to natural and anthropogenic activities, it is necessary to develop models reflecting changes in dissolved oxygen in relation to other water quality parameters.

This study was undertaken to investigate the regression relationships of surface and bottom dissolved oxygen levels with other water quality parameters in the Langat river estuary in Malaysia during the high tide. The other water quality parameters considered in this study were temperature, pH, salinity, conductivity, total ammonium nitrogen, nitrite+nitrate nitrogen, total phosphorus and chlorophyll *a*. Data collection of dissolved oxygen, other water quality parameters was done in five sampling stations along the estuary, and standard methods were used for water analysis.

A stepwise regression analysis was carried out using the S+ statistical software package. The results indicate that water quality parameters such as temperature, pH and total ammonium nitrogen were significantly related with surface dissolved oxygen levels. The model obtained was, Surface Dissolved Oxygen = 0.5521 temperature + 1.2814 pH - 4.2990 total ammonium nitrogen - 18.9033 with $r^2 = 0.6656$. On the other hand, the water quality parameters that were found to be significantly related to Bottom Dissolved Oxygen were pH, salinity, total ammonium nitrogen and Chlorophyll *a*. Specifically, the model obtained was for Bottom Dissolved Oxygen = 0.7861 pH + 0.2462 salinity + 0.1227 chlorophyll *a* - 4.5378 total ammonium nitrogen - 5.1514 with $r^2 = 0.8409$. In both models, all individual regression parameters were significant at the 5% level.

According to both models, there were positive correlation of dissolved oxygen with temperature, pH, salinity and chlorophyll *a* mainly due to natural phenomena. However, dissolved oxygen was negatively correlated with total ammonium nitrogen. As the main source for total ammonium nitrogen is human activities in upstream area, increase of this nutrient may cause the pollution reducing the available dissolved oxygen to the aquatic animals. Therefore, suitable actions should be undertaken to control the inputs of total ammonium nitrogen in this estuary to improve the ecosystem health.

LONG TERM CHANGES IN WATER QUALITY AND FISHERIES IN MUAR RIVER, MALAYSIA

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The Muar River is one of the longest rivers in Peninsular Malaysia, flowing 232km through the states of Negeri Sembilan and Johor, and draining a catchment of 6,200km². The river supports a significant level of economic activity, including fisheries, irrigation, navigation and water supply. Only 15% of the catchment consists of primary forest. The rest has been developed for agriculture and township development. The multiple use of the river coupled with the diversity of management regimes imposed by different state administrations and local governments have made the river a case study for integrated river catchment management.

Towards this end, a study was initiated to assess the current status and historical trends in water quality and fisheries of the river from 1992 - 2002. As with most Malaysian rivers, total dissolved solids (TDS) content progressively increased towards the river mouth, especially after Pergam village, undoubtedly due to seawater intrusion. However, suspended solids (SS) levels were higher at the potamonic zones of the river due land clearing activities. Dissolved oxygen fluctuated from 2.47 - 6.25 mg/l, most stations generally recording above 4.0 mg/l. However, there were some exceptions, especially Simpang Empat village, which is located close to the confluence with Merbudu river, which recorded the lowest value of 2.47 mg/l. The Merbudu river has been classified as polluted according to the Water Quality Index (WQI) for Muar River basin in 1998. The pH of the river water was generally acidic, a with a mean of 5.85, biochemical oxygen demand (BOD) levels ranged from 1.03 mg/l - 4.08 mg/l and chemical oxygen demand (COD) from 10.56 mg/l - 46.17 mg/l. The concentration of phosphate fluctuated within a range of 0.05 - 0.61 mg/l, rising towards the river mouth. Total ammonia nitrogen concentrations along the river exhibit a less definitive pattern, though levels ranged from 0.32 - 0.80 mg/l.

Only a limited number of fish species were found in the upstream sections of the Muar river, mainly small cyprinids such as the sia (*Mystacoulucus marginatus*) and terbol (*Osteochilus hasselti*), probably due to Ulu Muar and Kelinchi dams. Further downstream, fish diversity appeared to be dominated by rough species such as Clariids, Mystids and Channids. Also significant was the presence of the exotic Javanese carp (*Barbodes gonionotus*) and tilapia (*Oreochromis* sp) which have established permanently in the river. There is an actively inland fishery in the river, originally based on giant freshwater prawn (*Macrobrachium rosenbergii*). However, over the 1995 - 1998 period indicated that, while overall landings increased, prawn landings were declining. The increase largely came from finfish such as Mystids. However, tilapia has come to play an increasingly important role in the catch increasing by 2500% over the same period.

The study indicates that the river faces serious threats to its health where water quality is concerned and faces a potential decline in indigenous fish stocks due to displacement by exotics.

TOXIC CYANOBACTERIAL BLOOM AND THE OCCURRENCE OF MICROCYSTIN FROM FRESHWATER EUTROPHIC PONDS IN BANGLADESH

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Bangladesh is a tropical country of large alluvial plain with 1.3 million freshwater ponds and has a suitable environment for luxuriant growth of cyanobacteria. About 13 different species of cyanobacteria form blooms in eutrophic and hypertrophic ponds throughout the year but the bloom frequency is high during late winter to early summer and autumn. An observation was made on algal bloom formation in relation to some physico-chemical parameters and toxicity of bloom forming species in 12 ponds at three different localities in Bangladesh during March 1999 to February 2001. A bloom of *Microcystis aeruginosa* occurred in a pond at Matlab in Chandpur district. Methanol-water extract of bloom sample was analyzed by high performance liquid chromatography with UV detection and MS detected four types of microcystins viz., microcystin-RR, microcystin-YR, microcystin-LR and microcystin-LA. Another bloom of *M. aeruginosa* from Brahmanbaria district also contained microcystins. *Microcystis flos-aquae* that formed blooms in a pond at Dhaka was also analyzed but no microcystin or nodularin was found. Recently, an outbreak of *Anabaena* sp. in a culture pond caused mass mortality of cultured fish *Pangasius suchi* at Gazipur fish farm. Analysis of toxic components of the bloom sample is in progress. Toxicity and toxic components of other bloom forming cyanobacteria in Bangladesh remains to be identified.

FISH BLOOD AS POLLUTION INDICATOR IN AQUATIC ENVIRONMENT

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Aquaculture is a highly prospective and booming field among different arenas of agriculture. Cypermethrin is a widely used pesticide for cotton and brinjal pest and it is also used to control *Argulus* diseases in aquaculture. Carbofuran is another popular insecticide for paddy pest and famous for its role in aquaculture as piscicide. When these piscicide enter into the aquatic ecosystem both from external or internal sources, they jeopardize the total food chain through bioaccumulation and biomagnification. Human population also encounters various health hazards due to consumption of polluted fishes, prawns or through drinking water. Blood is an important path – physiological reflector and various indices of pesticidal pollution can be evaluated by studying the anomalies in blood parameters. In this study, red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) content, haematocrit (Hct) value, oxygen carrying capacity and glucose of *Labeo rohita* were taken as blood variables. Except for WBC count and glucose, both the pesticides elicited reduction in blood parameters, in comparison to control for all the variables. Carbofuran exhibited greater sensitivity in all concentrations and blood parameters than cypermethrin. Considering the aspect of aquaculture management, when pesticide treated fish were shifted to freshwater system, cypermethrin infected fish showed faster recovery than carbofuran. In our present study, we illustrated that the blood variables in fish can be used as pollution indicators of pesticide in aquatic environment by .

Table 1. Variations in some haematological parameters during exposure and recovery treatment in *Labeo rohita* on 10 % LC₅₀ concentrations of cypermethrin and carbofuran. Each value is the average performance of five separate observations. Means in the same column with different superscript are significantly different ($p < 0.05$).

Pesticide	Blood parameters	Exposed period			Recovery period		Rate of recovery (y)
		42	0 56		14	28	
Control		2.94±0.41	2.93±0.4	2.93±0.2	2.92±0.11	2.95±0.5	
Cypermethrin	TEC	2.30±0.31	3	1	2.75±0.31	2	0.006
Carbofuran	(1×10 ⁶ mm ³)	2.36±0.05	2.41±0.5	2.78±0.3	2.84±0.04	2.96±0.4	0.004
			2	2		3	
			2.31±0.0	2.82±0.0		2.95±0.0	
			7	3		5	
Control		18.46±2.61	18.50±2.	18.54±2.	18.49±2.3	18.55±1.	
Cypermethrin	TLC	20.23±3.21	61	11	1	89	0.019
Carbofuran	(1×10 ⁶ mm ³)	20.42±0.46	20.08±3.	19.09±3.	18.79±2.6	18.65±1.	0.017
			61	30	1	75	
			22.11±0.	19.11±0.	18.99±0.4	18.62±0.	
			17	30	1	23	
Control		30.5±0.21	31.0±0.2	31.00±0.	30.5±0.21	30.7±0.2	
Cypermethrin	Hct (%)	25.40±0.80	2	20	29.5±0.72	1	0.067
Carbofuran		24.50±0.96	26.70±0.	28.90±0.	29.5±1.15	30.8±0.7	0.085
			71	75		1	
			25.80±0.	28.40±1.		30.8±0.8	
			95	17		5	

MITIGATING ENVIRONMENTAL IMPACT OF CAGE CULTURE THROUGH INTEGRATED CAGE-CUM-COVE CULTURE SYSTEM IN TRI AN RESERVOIR OF VIETNAM

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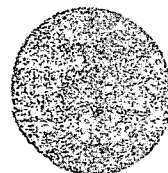
A study was conducted at Truong Dang Cove of Tri An Reservoir from June 2003 to June 2004, to assess the feasibility of the integrated cage-cum-cove system, to assess effects of cages on water quality and plankton abundance in both cove and the main reservoir. The study also assesses the effects of cages on the fish production in coves, and the effects of accumulated wastes on cove bottom on terrestrial vegetation.

Five 16-m³ net-cages were set up at the middle of the cove for the culture of red tilapia (*Oreochromis niloticus* x *O. mossambicus*) stocked at 50, 75, 100, 125 and 150 fish m⁻³ from 19 August 2003 to 17 January 2004. Water samples were collected at 20 sampling stations grouped into 6 locations with different distance to the cage sites for water quality analyses. Ten plots (1 x 1 m quadrat) were selected for sampling sediment and terrestrial vegetation, with five plots at the cage culture sites and an another five at random sites before and after establishment of cage culture to determining vegetation biomass and analyzing contents of nitrogen, phosphorus and organic matter.

The results of this study showed that red tilapia grew well in cages, and cage wastes might have enhanced the growth performance of open-water fish in cove. The study also showed that nutrients released from cages to the cove did not significantly affect the water quality in the main reservoir, and no significant accumulation of cage wastes on the cove bottom. The results imply that the fish in the cove and terrestrial vegetation during the dry season may efficiently utilize the nutrients derived from the cage culture.

The integrated cage-cum-cove culture system can enhance fish production in coves through cage cultured fish and increased production of open-water fish by reusing wastes from cage culture. The integrated cage-cum-cove can use appropriate stocking composition in open water to efficiently reuse cage wastes, use the terrestrial vegetation to reuse the cage wastes at the cove bottom. In addition, the system can also reuse the dry period of the cove to decompose organic cage wastes. However, cage culture in cove is a new practice, and needs more research to develop an environmentally-sound integrated cage-cum-cove culture system.

***SS Improve Resource Use Efficiency
in Asian Integrated Pond-dike Systems***



SEDIMENT ACCUMULATION IN FISH PONDS: ITS QUANTITY AND QUALITY AS A CROP FERTILIZER

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Accumulation of nutrient rich sediment in aquaculture pond bottoms has been reported. The accumulated sediment however fills ponds reducing their volume while accumulated organic matter releases toxic elements (hydrogen sulfides and nitrites) and impacts a high oxygen demand. Periodic removal of accumulated sediment is thus necessary. The nutrients trapped in the accumulated sediment however could be a useful on-farm resource. Utilization of nutrients in pond sediment in fertilization of agricultural produce in Asia has had positive impacts on the rural communities through reduced costs of fertilizers and increased income levels due to diversity in produce. The adoption of integrated aquaculture – agriculture systems in Africa where serious food challenges exist and lie a head, has been slow. Its promotion has been limited by a scarcity of quantitative data on the potential benefits of one system to the other. The aim of this study was to quantify the sediment and nutrients accumulating in pond sediments during a culture period, determine whether accumulation is affected by nutrient input type and stocking density and assess its potential fertilizing effect.

Sixteen 200m² earthen ponds were allocated 4 treatments with 4 replicates in a completely randomized 2x2 factorial design. In all ponds 20g Nile tilapia fingerlings were stocked. The factors analysed were input type and stocking density. The input types used were 50kg dm ha⁻¹ day⁻¹ of chicken manure and a 25% protein diet fed at 3% body weight day⁻¹. Stocking densities tested were 1 and 2 fish m⁻². Ceramic tiles of 0.5 by 1m² were installed horizontally at 5cm depth in the sediment before starting the experiment. At monthly intervals core sediment samples were taken above the tiles to measure nutrient and sediment accumulation during the experiment, as well as changes in bulk density.

Sediment accumulated at a rate of 454 -533 tons ha⁻¹ year⁻¹ and contained 625 – 1015 kg of nitrogen which could potentially supply the required nitrogen fertilizer dose in Egypt for a 2 – 3.5 ha of corn, resulting in the production of 12 to 21 tons of maize. The deposition of sediment or nutrients during the production cycle was not affected by nutrient input type or stocking density. Field trials on the fertilizing effects of pond sediments on the main agricultural crops in the African farming systems would yield further information that would go along way in promoting the adoption of integrated aquaculture-agriculture systems in Africa.

**POND-DIKE SYSTEMS AND LIVELIHOOD IN RURAL AND PERI-URBAN AREAS OF
MYMENSINGH DISTRICT OF BANGLADESH - A REVIEW THROUGH PCA
(PARTICIPATORY COMMUNITY APPRAISAL) METHOD**

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Links between the pond and surrounding land for horticulture is a distinctive feature of farming households in Bangladesh. Rural and peri-urban settlements in Mymensingh District, Bangladesh were selected for a community-level assessment of the importance and role of pond-dike systems. A total of 12 participatory, group-based activities were used with 200 male and 120 female participants from four communities over a five day process in each community. Focus groups were selected on the basis of local perceptions of overall well-being, and gender and the characteristics of their pond-dike systems. These preliminary findings were cross-checked in subsequent meetings with four stakeholder groups: representatives of households from the communities, traders, NGOs, and government agencies and researchers.

The effects of gender, well-being and location on farmers' regular activities and food consumption patterns were identified. Fish was preferred by all groups, but his preference was strongest among poorer women in peri-urban communities. Fish culture was equally important as an enterprise among richer and poorer men, whereas vegetable cultivation was more important to men than women but wealth and location also affected its importance. Lack of knowledge was a particular problem to farmers growing fish and vegetable in the rural areas. Fish disease, high price of input, lack of money were also identified as constraints by fish producers. Expected use and current use of ponds, problems and benefits associated with fish culture were also found to be affected by groups emphasizing vegetable, orchard and fish culture within their systems. The role of pond for family use, which was a major objective for pond construction, was found to be significantly different between rural and peri-urban areas. Fish culture is now the dominant use of ponds for households irrespective of their focus on vegetable, orchard or fish production and they are utilized less for general domestic use. Ponds are relatively more important as a source of irrigation water in rural than in peri-urban communities. It was concluded that considerable potential exists for further integration and development of pond-dike systems, which could contribute towards improved livelihoods of both better off and worse off people.

COMPARISON BETWEEN LOW INPUT AND HIGH INPUT INTEGRATED POND-DYKE AQUACULTURE SYSTEMS IN SOME VILLAGES OF MUKTAGACHA, BANGLADESH

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An on-farm study was conducted during July 2002 to February 2003 under EC funded POND LIVE project to compare two major existing integrated pond-dyke production systems in Muktagacha, Mymensingh area of Bangladesh. Three farmers practising carp polyculture managed as low input system and other three practising *Pangasius* sp. monoculture managed as high input system was selected from two neighbouring villages, Noshirpur and Bashati of Muktagacha Upazilla under Mymensingh district. The input-output of both categories of farming systems were computed in terms of biomass (Kg/ha), monetary value (cash and non-cash Taka/ha) and nutrient (Kg Nitrogen/ha). In low input system averages of pond and dyke inputs were 9,235 Kg/ha and 6,553 Kg/ha, respectively with the averages of outputs of the same were 1,526 Kg/ha and 7,788 Kg/ha, respectively. Whereas, in case of high input system the averages of pond and dyke inputs were 72,818 Kg/ha and 7,135 Kg/ha, respectively with the averages outputs of the same were 32,460 Kg/ha and 6,971 Kg/ha, respectively. These pond dyke inputs and outputs were converted into cash Taka as well as non-cash Taka. The averages of pond inputs in Kg N were 74 Kg N/ha in low input system and 2,025 Kg N/ha in high input system. The averages of pond outputs in Kg N in low and high input systems were 36 Kg N/ha and 690 Kg N/ha, respectively. Significant difference ($P < 0.001$) was found between pond inputs in Kg biomass and Kg N of low input system and high input system. Pond outputs in Kg biomass, cash Taka and Kg N of low and high input systems were also significantly different.

Table 1 Input-output status (mean value $\pm$ S.E.) in low and high input systems

Parameters	Low input systems	High input systems	Level of significance
Pond input in Kg/ha	9,235 $\pm$ 3,019	72,818 $\pm$ 5,160	($P < 0.001$)
Pond input in Cash Taka/ha	18,542 $\pm$ 5,846	641,243 $\pm$ 58,485	($P < 0.001$)
Pond input in Non-cash Taka/ha	15,752 $\pm$ 9,431	459,631 $\pm$ 47,998	($P < 0.001$)
Pond input in Kg N/ha	74 $\pm$ 17	2,025 $\pm$ 77	($P < 0.001$)
Dyke input in Kg/ha	6,553 $\pm$ 487	7,135 $\pm$ 160	($P > 0.05$)
Dyke input in Cash Taka/ha	3,130 $\pm$ 1,187	3,533 $\pm$ 1,036	($P > 0.05$)
Dyke input in Non-cash Taka/ha	3,934 $\pm$ 664	3,598 $\pm$ 72	($P > 0.05$)
Pond output in Kg/ha	1,526 $\pm$ 230	32,460 $\pm$ 574	($P < 0.001$)
Pond output in Cash Taka/ha	48,903 $\pm$ 4,934	1303,760 $\pm$ 64,829	($P < 0.01$)
Pond output in Non-cash Taka/ha	19,617 $\pm$ 11,461	13,691 $\pm$ 6,069	($P > 0.05$)
Pond output in Kg N/ha	36 $\pm$ 4	690 $\pm$ 31	($P < 0.001$)
Dyke output in Kg/ha	7,788 $\pm$ 2,441	6,971 $\pm$ 4,196	($P > 0.05$)
Dyke output in Cash Taka/ha	37,522 $\pm$ 18,072	54,415 $\pm$ 30,314	($P > 0.05$)
Dyke output in Non-cash Taka/ha	33,328 $\pm$ 11,129	10,545 $\pm$ 6,138	($P > 0.05$)

SEDIMENTATION AND NUTRIENTS BUILD UP IN TILAPIA PONDS UNDER DIFFERENT INPUT SYSTEMS

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An experiment was conducted in nine 200 m² earthen ponds using randomized complete block design at the Asian Institute of Technology, Thailand during November 2003 to April 2004 in order to determine the rates of sedimentation, OM accumulation, and nutrient build up in sediment in tilapia ponds stocked at 2 fish.m² under different pond inputs. There were three treatments in triplicate: inorganic fertilizer (IF), inorganic fertilizer with chicken manure (IF+CM), and inorganic fertilizer with feed (IF+Feed). Chicken manure was applied at the rate of 140 kg ha⁻¹ w⁻¹ to three ponds with urea and triple super phosphate to give a total amount of 4 kg N and 1 kg P ha⁻¹ d⁻¹ to all ponds. Pelleted feed (30 % protein) was applied to ponds daily at the rate of 5 %, 3 % and 1 % body weight of fish for the first, second and last two months, respectively.

Depth and rates of sedimentation, and OM accumulation significantly varied among treatments, and between PVC at pond bottom and PVC at 30cm above from bottom, only with the exception for OM accumulation between IF and IF+CM treatments, which is not significant. The treatment IF+Feed, and PVC at bottom provided the highest values for depth, rates of sedimentation and OM deposition.

The effects of treatments on water alkalinity, NH₃, and chlorophyll- a were found significant, while on TKN, NO₂, TP and SRP were not significant. Significantly higher values were observed for alkalinity in IF+Feed, NH₃ in IF+CM, and chlorophyll-a both in IF+CM and IF+Feed treatments than that in other treatments. Significant effects of treatments on sediment quality were found for OM, Cu and Zn, while on total-N, total-P, K, B, Fe, and Mn the effects were not significant. Significantly higher levels of OM were found in IF+CM and IF+Feed treatments, and both Cu and Zn were found in IF+CM treatment. The correlation between total-N in sediment and TKN in water were significant in all treatments. Regression analysis indicated that all the parameters of sediment quality except K and B significantly increased over the 6-months culture period. Nitrogen and P data of top 5 cm layer of sediment at final sampling revealed that the fertilizer values of sediment of 1 ha pond area were 1.48-1.66 t urea and 0.21-0.26 t TSP.

THE NUTRITIVE VALUE OF TILAPIA POND WATER AND SLUDGE FOR CHILLI CULTIVATION

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A plot trial was carried out at the Asian Institute of Technology, Thailand to assess the fertility value of pond water and sediments from tilapia pond for chilli cultivation. Four 200 m² earthen ponds were stocked with tilapia at a rate of 2 fish.m². Ponds were fertilized with urea and triple super phosphate (TSP) at rates of 24 kg N and 1 kg P ha⁻¹ week⁻¹. Twenty four chilli plots (2.0 x 0.6 m) were constructed on pond dikes, and six treatments viz.: (1) control with no sludge and fertilizers (S₀), (2) application of sludge one time a week (S_{1/wk}), (3) two times a week (S_{2/wk}), (4) one time for two weeks (S_{1/2wk}), (5) application of sludge + half of the recommended dose of phosphorous (P) + recommended dose of N and K (S_{1/2wk} + N₁P_{0.5}K₁), and (6) recommended doses of N, P and Ks (N₁P₁K₁), were randomly assigned. There were four replicates. The sludge (162 g dry weight) and water (36 liters) from tilapia ponds collected into a tank using a submersible pump and applied to individual chilli plots according to the above treatments. Plots were irrigated daily, except during rainy days.

Chemical analysis of sludge showed that it contained 67 mg kg⁻¹ NH₄⁺-N and NO₃⁻-N, 97 mg kg⁻¹ available P, and 41 mg kg⁻¹ exchangeable K, and pond water contained 2.81 mg l⁻¹ NH₄⁺-N and NO₃⁻-N, 0.19mg l⁻¹ soluble reactive P, 0.48 mg l⁻¹ total P, and 1.55 mg l⁻¹ exchangeable K.

The pod yield, plant biomass (excluding pods), and plant height of chilli were significantly higher in treatments S_{1/2wk} + N₁P_{0.5}K₁ (Treatment 5) and N₁P₁K₁ (Treatment 6) than the rest, and also there were significantly higher removal of N, P and K by Chilli pods and leaves in both treatments. There were no significant differences in growth and yield responses of chilli between the control and sludge treated plots. The fresh yields of chilli were 17,039 kg and 16,284 kg.ha⁻¹ in treatment 5 and 6, respectively. The results indicated that water and sludge from fertilized tilapia ponds have a potential for supplementing P requirement of chilli plants under controlled on-station research conditions. However, on-farm research taking into consideration of both economics and chilli yield is necessary before arriving at a final conclusion.

FOOD PREFERENCE OF COMMON CARP *Cyprinus carpio* AND ROHU *Labeo rohita* IN MONOCULTURE AND POLYCULTURE: SHIFTS IN GRAZING ON PLANKTON UNDER FED AND UNFED CONDITIONS

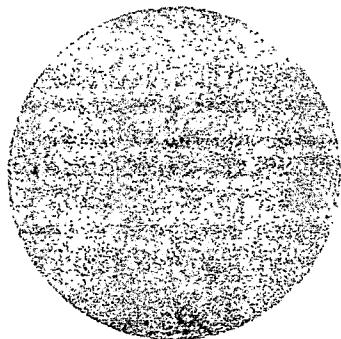
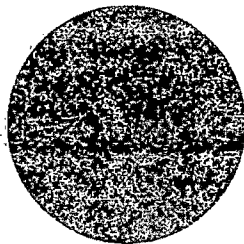
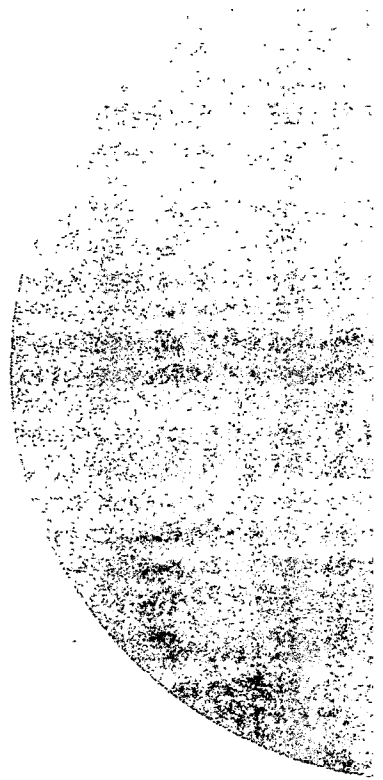
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Understanding of food and feeding habit of fish is very important for their efficient management. Rohu (*Labeo rohita*) is known as a column feeder mainly feeding on plankton and common carp (*Cyprinus carpio*) is a bottom feeder mainly feeding on benthos in the natural system. These fishes are also very dependant on supplementary feeds. So, food and feeding habits of these species can be changed with food and feed availability in the system. An experiment was conducted to assess the effect of common carp and supplementary feed on the food availability in the pond and to compare the food and feeding habit of *L. rohita* alone or with *C. carpio* in fertilized or in fertilized and fed ponds. There were six treatments in triplicate. The treatments were: *L. rohita* (1.5 m⁻²) alone with and without supplementary feed, *L. rohita* (1.5 m⁻²) plus 0.5 m⁻² *C. carpio* with and without supplementary feed, and *L. rohita* (1.5 m⁻²) plus *C. carpio* (1.0 m⁻²) with and without supplementary feed. It was found that overall phytoplankton, zooplankton as well as benthos biomasses were higher in treatments with feed than the treatments with no feed. *L. rohita* mainly fed on more phytoplankton than zooplankton when there was no supplementary feed but in presence of supplementary feed it preferred to ingest more zooplankton than phytoplankton. Effect of *C. carpio* was significantly higher in treatment with *C. carpio* than the treatment with no *C. carpio* in terms of food availability in water and food ingestion. Ingestion rate was higher in presence of 0.5 m⁻² *C. carpio* than 1.0 m⁻² *C. carpio*. *C. carpio* fed on more benthic organism than phytoplankton and zooplankton but in presence of supplementary feed it shifted its food habit partially from phytoplankton, zooplankton and benthos to artificial feed. These shifting tendencies may also be affected by the quality of feed and environmental condition.

Posters



INVESTIGATION ON THE FEEDING REGIME OF SEVEN-BAND GROUPER *Epinephelus septemfasciatus* LARVAE USING DIFFERENT ROTIFER SIZES

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The seven-band grouper *E. septemfasciatus* is one of the new target species for stock enhancement and aquaculture in Japan, because of its high commercial value and low catch in the wild. Seedling production of this species has already started, however, mortality is high during early larval stage, partly due to lack of knowledge regarding its feeding habits. This study aimed to investigate the feeding characteristics (food selectivity and diurnal feeding) and to improve the feeding regime of seven-band grouper larvae.

Rotifer, *Brachionus plicatilis* (L-type, lorica length: 119-241 μ m) and *B. rotundiformis* (S-type, 93-179 μ m; SS-type, 90-159 μ m), were fed from the first feeding (4 days after hatching; day 4) until day 20. Larvae and rotifers were sampled on days 4, 9, 13, 15, 17, 20, respectively. Standard length (SL) of each sample was measured, and the number and lorica length of rotifers in the gut of each larva were counted and measured. Lorica length of rotifers in the rearing tank was also measured. Food selection among different sizes of rotifers (for 20 μ m size classes) was calculated using Chesson's α -index. Larvae ranging from 2.1-3.0 mm SL preferred SS-type and S-type rotifers (100-160 μ m), while larvae ranging from 3.0-4.0 mm SL preferred 120-180 μ m rotifers. Larvae bigger than 4.0 mm SL preferred over 160 μ m rotifers. We concluded that small-sized rotifers (SS-type, S-type) are necessary during the first feeding (<3.0 mm), while L-type rotifer is essential for larvae bigger than 3.0 mm SL.

Based from this result, we investigated the effect of different feeding regimes on survival, growth, and quality of larvae. Three feeding treatments (SS, L and SS-L tank) were prepared. SS tanks were fed SS-type rotifer solely from the first day of feeding (day 4) until day 20. L tanks were fed L-type rotifer only throughout the experiment, while SS-L tanks were fed SS-type rotifer from day 4 until day 10 (<3.0 mm SL), and then fed L-type rotifer from days 11 to 20. Larvae were sampled on days 4, 7, 11, 13, 17, 20 and SL was measured, and the number of rotifers in the gut was counted. Furthermore, the diurnal feeding activity on days 6-18 was also investigated. We reared larvae under natural light condition, and fed SS-L rotifer. Sampling was done at 4h interval for 24h. The number of rotifers in the gut was counted, and enzyme activity analysis was done on day 6, 12 and 18. On day 21, the quality of the larvae was evaluated. Larvae from each tank were transferred to another small tank, and survival 1 hour after transfer was determined. Docosahexaenoic acid (DHA) content of the fish in day 21 was measured.

The number of rotifers in the gut of larvae during initial feeding (day 4) was as follows: 3.2 ind. (SS tank), 0.2 ind. (L tank), and 4.0 ind. (SS-L tank). Feeding incidence was also higher when larvae were fed with SS-type rotifers. Survival of larvae on day 20 was 24.2%, 7.2%, and 33.6% in SS, L, and SS-L tanks, respectively. SL of larvae on day 20 was as follows: 4.1 mm (SS tank), 3.8 mm (L tank) and 4.3 mm (SS-L tank). Larvae fed only during light condition and enzyme activity was highest in the day time. DHA composition and survival 1 hour after transfer of larvae in SS-L tank were higher than other feeding treatments.

These results demonstrated that survival, growth, feeding incidence and quality of larvae can be improved when fish were fed with the feeding regime based on its feeding selectivity.

COMPARATIVE STUDIES ON THE EFFECT OF DIMETHYLSULPHOXIDE, GLYCEROL AND METHANOL AS THE CRYOPROTECTANTS IN SPERM CRYOPRESERVATION OF GREEN RIVER CATFISH *Mystus nemurus*

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Studies on the effect of dimethylsulphoxide, glycerol and methanol as cryoprotectants in sperm cryopreservation of green river catfish, *Mystus nemurus* were conducted to determine the motility, fertility and hatching rate using cryopreserved sperm.

The results showed that the undiluted sperm of *M. nemurus* has large number of spermatozoa ranging from $1.5 \times 10^7 \text{ ml}^{-1}$ to $3.0 \times 10^7 \text{ ml}^{-1}$. The sperm at low temperature could survive much longer than at higher temperature. The percentage of motile sperm diluted in six extenders showed no significant difference ($p > 0.05$). Further studies of $6.5 \times 10^6 \text{ ml}^{-1}$ of *M. nemurus* sperm were done to observe the efficiency of cryoprotectants. Sperm that were diluted in 100% extender without addition of cryoprotectant became immotile as soon as the samples were stored in liquid nitrogen container (-196°C). Glycerol is the best cryoprotectant for *M. nemurus* sperm cryopreservation. However, it was influenced by the concentrations used. Ten percent glycerol showed the best result. The determination of sperm motility, fertilization rate and hatching rate is important to identify the effectiveness of the cryoprotectants. All concentrations of cryoprotectants tested, showed significant differences ($p < 0.05$) in sperm motility.

**EFFECTS OF WATER TEMPERATURE AND PH ON THE SURVIVAL AND GROWTH OF
MARBLE GOBY LARVAE *Oxyeleotris marmoratus* (BLEEKER)**

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Studies on the effect of different water temperatures and pH on the early larval stage of marble goby *Oxyeleotris marmoratus* were conducted. The best growth was observed in the larvae reared at a temperature of 30°C, with the final total length and the mean growth rate of 4.23mm (± 0.20) and 0.12 mm.day⁻¹ respectively. The growth of larvae was significantly the lowest in larvae reared at a temperature of 24°C (0.05 $\pm$ 0.005 mm.day⁻¹). Larvae reared at the temperature of 24°C and 26°C were significantly lower ($P < 0.05$) in survival rates than those reared at 28°C and 30°C, mean survival rates at those temperatures were 16.67% (± 8.00) and 18.00% (± 5.11), respectively. The survival rate of larvae at 32°C (22.8 $\pm$ 5.11) was not significantly different compared to those of larvae at 30°C and 26°C. The exposures in different ranges of pH (4, 5, 6, 7 and 8) were studied. After 96 hours of non-feeding treatments, highest survival was observed at pH 6 and 7 with the survival rate of 22.67% and 23.67% respectively. Larvae that exposed to pH 6 and pH 7 were significantly lower ($P < 0.05$) in survival rates than those at pH 4, 5 and 8.

**PARTIAL REPLACEMENT OF ARTEMIA SALINA BY *Moina* sp. IN THE REARING OF
EARLY POSTLARVAE OF THE SHRIMP *Litopenaeus schmitti***

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The objective of this study is to assess *Moina* sp. as feed for early postlarvae cultures of shrimp *Litopenaeus schmitti*. Different diets using *Moina* sp. were designed and their influence on postlarval quality were evaluated. Additionally, the enzymatic activity of amylase and protease, as well as soluble protein in postlarvae, were characterized. Postlarval groups fed on *Artemia* and *Artemia/Moina* sp. had a significantly greater growth than those fed on *Moina* sp. and *Moina* sp./microparticulate. No differences were observed in osmotic stress resistance, where the survival percent of stressed postlarvae was above 91%. Postlarval moisture showed no differences with values ranging from 78.1-83.6%. Diets with *Artemia* or *Artemia / Moina* sp. were found to have better nutritional levels. Diets with *Moina* sp. and *microparticulate/Moina* sp. had low carbohydrate levels, and at some stages they were poor digestibility or protein content. To our knowledge this is the first report on the use of *Moina* sp. as feed for *L. schmitti* early postlarvae.

MOBILE HATCHERY: A NEW TOOL FOR FISHERIES EXTENSION

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A mobile hatchery has been developed under the AIMS Thailand sub-component since 2002 to use as a tool for aquaculture extension in rural areas where there is no access to regular hatcheries. However, the mobile hatchery has proven to be more flexible than it was designed to be. It has been used to breed wild fish in the field where transportation of the fish to the main hatchery is impossible. It has been demonstrated in Nakhonphanom, Udonthani, Mukdahan and Nam Houm (Lao PDR). A hatching hapa measuring 2x2x0.5 m with flow-through water supply can be used to hatch 3.4x10⁶ eggs of *Barbodes gonionotus*, with 93% fertilization rate and 86% hatching rate, 1.5x10⁶ eggs of *Labeo rohita* with 74% fertilization rate and 95% hatching rate, or 1 x10⁶ eggs of *Cirrhinus microlepis*, with 60% fertilization rate and 30% hatching rate. Funnel-type incubators with 15 liter volume can be used to hatch 1.0x10⁵ eggs of *Probarbus jullieni* with 60% fertilization rate and 40% hatching rate. It also has been used for fisheries conservation awareness rising. This paper presents details the use of the mobile hatchery for fisheries development in Thailand.

LARVAL DEVELOPMENT OF *Chicoreus ramosus* (LINNAEUS, 1758) (GASTROPODA: MURICIDAE) IN MUKAHEAD MARINE RESEARCH STATION, PENANG

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The culture method and larval development of *Chicoreus ramosus* (Linnaeus, 1758), collected from Pulau Segantang, Kedah, are illustrated. Broodstocks were collected from the wild and kept in hatchery using 1 ton fiberglass tank supplied with continuous flowing seawater and local rock oysters as food. The broodstocks were kept in the hatchery until they spawned naturally. An adult sized 234mm in length and 186mm in width laid 356 capsules in a single egg mass in 10 days. The hatching duration was 8 days. The egg capsules were transferred into separate fiberglass tank with ultrafiltered (1µm) seawater and the seawater was changed every alternate day. The colour of the egg capsules changed from pale yellowish indicating the development of the embryos. On average, 548±54.4 embryos were observed in each capsule but only 76.78% hatched and developed to planktotrophic larvae.

The larvae were sieved onto a 250 µm mesh size sieve and transferred into 500L cylindrical fiberglass tank supplied with aeration. The larvae were fed with phytoplankton *Chaetoceros calcitrans* and *Isochrysis galbana*. The morphology of the shell and soft body of the larvae were observed daily. The larvae reached metamorphosis stage when a pronounced beak was observed on the outer lip of the larvae shell. The pronounced beak appeared between the 11th and the 13th day after hatching. When the pronounced beak was observed on the shell, the mantle of the larvae was actively touching the surface of the culture tank. Hard substrates with barnacles and oyster spats were prepared as settling surface for the metamorphosed juveniles.

IN VITRO INCUBATION OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* EGGS

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An experiment was carried out to determine the *in vitro* incubation of giant freshwater prawn, *Macrobrachium rosenbergii* eggs. Wild broodstocks of giant freshwater prawn kept in freshwater were fed with squid and a commercial diet, spawned successfully in fibreglass tanks. A sexual ratio of 1 male: 4 female was used where 60 pieces of female resulted in 55% moulting and 48.3% spawning rate. Spawned eggs showed normal development with 82.62% ripe eggs compared to 17.36% dropped eggs due to incomplete fertilisation. Fertilised eggs were removed from the brooding chamber after 5 days of spawning and 200 pieces were incubated in each round glass beaker at a constant temperature of $30^{\circ}\text{C} \pm 0.5$ with gentle aeration. Eggs incubated at salinity 0 ppt, 2 ppt, 4 ppt, 6 ppt, 8 ppt, 10 ppt, 12 ppt, 14 ppt, 16 ppt, 18 ppt and 20 ppt produced normal newly hatched larvae after 19 days. Hatching percentage were 90.76 ± 7.26 , 92.03 ± 0.85 , 92.03 ± 2.22 , 92.92 ± 2.59 , 90.66 ± 4.61 , 92.00 ± 3.44 , 94.83 ± 1.76 , 95.18 ± 0.63 , 94.32 ± 4.50 , 95.37 ± 5.18 , and 90.45 ± 0.55 , respectively with no significant difference for the salinity range of 0 – 20 ppt. At the higher salinity of 25 ppt with a hatching percentage of 92.73 ± 3.51 , the eggs showed normal development but produced $19.83\% \pm 1.53$ abnormal larvae and this was significantly different compared to those from the lower salinity. At 30 ppt salinity the eggs failed to hatch due to inability to survive at the eye pigment stage. Hatching occurred on two consecutive days in all salinities tested. Larvae from both hatching day are morphologically similar in term of total length and body weight and no significant difference except for abnormal larvae.

DEVELOPMENT OF DIGESTIVE TRACT IN HYBRID CATFISH

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Histological development of digestive tract was studied in hybrid catfish from hatching (day 0) until day 7. Specimens were obtained from interspecific hybridization between *Clarias macrocephalus* female and *C. gariepinus* male and maintained in hatchery ($28 \pm 1^\circ\text{C}$). The digestive tract of newly hatched larvae was a simple undifferentiated tube. One day after hatching (1 dAH), the mouth started to open and the gut was differentiated into a buccal cavity, oesophagus and simple digestive tube. The formation of pancreas and liver was distinguished as a clump of cells. On the 2nd dAH, the development of gastro-intestinal tract had started and development of zymogen granules in pancreas was observed. When the larvae started their exogenous feeding on newly hatched *Artemia* nauplii (3rd dAH), the stomach developed with isometric epithelium cells and the intestine was well developed and divided into three sections. By 5th dAH the development of gastric gland was observed beneath the gastric epithelium and the clearly visible PAS-positive mucus layer in the luminal side of the stomach epithelium indicated that their stomach had attained its typical morphological characteristics and was fully functional.

**ONTOGENY OF THYROID HORMONES DURING EARLY DEVELOPMENT STAGE OF
MARBLE GOBY *Oxyeleotris marmoratus* (BLEEKER) LARVAL**

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Thyroid hormone is considered to play important roles in the regulation of development and growth of larval fish. In this regards, a study was carried out on the structural and functional development of the thyroid gland in the marble goby's early life stages. Larvae were collected at incremental time intervals from newly hatched to 30 days after hatching (dAH). Thyroxine (T4) immunostaining was used to assess the development of thyroid follicles and the Elisa kit was used to quantify T4.

It was found that at hatching (0dAH) T4 was already detected in the larvae even if the thyroid follicles were not yet developed. T4 levels decreased from 3 – 9 dAH then increased again from 10 – 30 dAH. Since the thyroid follicles were not yet developed at hatching (0dAH), the T4 present at hatching was most probably not produced by the larvae itself but are, most probably, of parental origin. T4 levels decreased thereafter at the larvae utilized their T4. The increase in T4 at 10 dAH indicated that the larvae was already producing its own T4 as fully developed thyroid follicles were detected by histological examination at 13 dAH.

DIFFERENT FEEDING REGIMES AFFECTING CARCASS TRAITS IN TRIPLOID AND ALL-MALE POPULATIONS OF NILE TILAPIA

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To avoid the problem of stunting, production of all-male and triploid stocks are methods in Nile Tilapia (*Oreochromis niloticus*) aquaculture to increase the overall productivity. All-male populations are known to facilitate culture in a broad range of systems as they show better growth performance and homogeneity if compared to mixed sex populations. Due to their functional sterility the use of triploid seed represents an effective approach to eliminate negative effects of uncontrolled reproduction. Although triploid fish show various advantages, especially females tend to produce large amounts of intestinal fat if subjected to *ad libitum* feeding regimes. Fodder could be saved if the energy, deposited in intestinal fat stores, would be utilised for somatic growth. Therefore an eight week feeding trial during the early growth period was conducted to investigate the effects of different feeding regimes on the body composition of three different genetic groups of tilapia (Lake Manzala strain, Egypt: mixed sex, triploid, all-male) with emphasis on intestinal fat deposition.

Three month old tilapia of the different genetic groups, stocked in replicates of 26 fish in 80 l-aquaria in a recirculating system, were either fed *ad libitum* three times a day or subjected to food deprivation every second day. At five month of age, all fish were slaughtered and carcass traits were measured. Males fed *ad libitum* had a 60 % better growth compared to the restricted groups. The all-male groups showed the best overall growth capacity. The growth of females in the restricted regime was 92% inferior to the growth of their daily fed counterparts. Food deprivation did not result in decreased amounts of intestinal fat in the triploids, neither in males nor in females, during the early fattening phase under the given feeding regimes. Only in the all-male groups intestinal fat was significantly affected by food restriction.

**A NEW YY-MALE OREOCHROMIS NILOTICUS STRAIN ORIGINATING FROM AN
EXCEPTIONAL MITOTIC GYNOGENETIC MALE - LINE DEVELOPED AND GROWTH
PERFORMANCE TESTING OF THE GENETICALLY ALL MALE PROGENIES**

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As basis for the development of so called yy-males, producing all male offspring if mated with normal *Oreochromis niloticus* females, an exceptional *O. niloticus* male was used which was obtained by artificially induced mitotic gynogenetic reproduction and known to produce only all male offspring. In a first step diethylstilbesterol (500mg/kg of feed) was orally administered for 40 days to first feeding progenies from this evidently homozygous yy-male and normal females (xx) (Lake Manzala population, Egypt). The resulting individuals were functional females but possessed a genetically male (xy) status. Progenies of these functional females (xy) with normal males (xy) resulted in approx. 25% yy-males, which were verified by progeny sexing. Only males, which produced 100% male progenies (n>50) with a random sample of at least three normal females were designated to be yy-males. For the easy continuation of the yy-male status in a breeding strain, yy-females were needed. To obtain yy-females yy-males were mated with functional females (xy), their progenies (approx. 50% of them were genetically yy-males) were changed to functional females by diethylstilbesterol enriched feed and the needed yy-females were verified by progeny sexing. By mating yy-males with yy-females yy-male offspring was produced in large quantities. To get first information about the growth potential of the new yy-male strain originating from the above mentioned homozygous yy-male, performance comparisons between all male groups (progenies of yy-males mated with normal females (xx)) and traditional mixed sex groups were conducted in 80 l-units. The all male groups were 12.4% larger if compared with the whole mixed sex groups but 2.5% smaller than the males within the mixed sex groups.

GENETIC DIVERSITY OF SEBASTES INERMIS BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) AND MITOCHONDRIAL DNA ANALYSIS

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Genetic diversity of dark-banded rockfish (*Sebastes inermis*) in wild and cultured stocks was examined by random amplified polymorphic DNA (RAPD) and mitochondrial DNA (control region) polymorphism analysis. Total of 46 polymorphic fragments were produced from 9 random primers, OPA-01, OPA-11, OPB-08, OPB-20, OPE-05, OPG-10, OPH-12, OPI-04 and OPI-20, with 7, 3, 6, 20, 6, 6, 10, 4 and 5 genotypes, respectively. Among OPB-08 depended polymorphic bands, 300 bp fragment appeared only in wild population at 61.3% frequency level, and suggested that this RAPD would be a useful marker for the discrimination of fish stock, wild or cultured, due to an effective parent number of each stock. In restriction fragment length polymorphism (PCR-RFLP) analysis of mtDNA control region with *Mbo* I and *Xsp* I, 8 mtDNA composite haplotypes were observed with different haplotype frequency and stock-specific haplotypes. It is expected that RAPD marker and mtDNA haplotypes identified in this study would be used as effective genetic monitoring tools in our ongoing stock enhancement program of Korea.

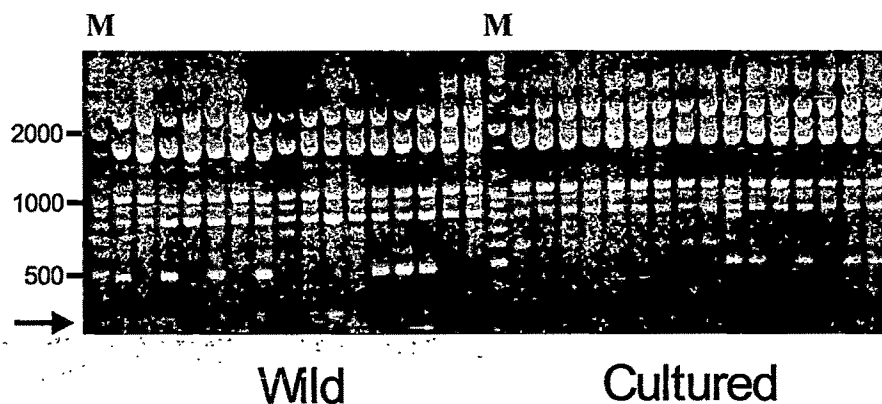


Figure 2 RAPD pattern for dark-banded rockfish, *Sebastes inermis* using random primer OPB-08. A 100bp Plus DNA Ladder was used as a DNA marker (lane M). An arrowhead indicates a 300 bp RAPD marker observed in the specimens from wild stock, but not in the cultured stock.

PHYLOGENETIC RELATIONSHIP BETWEEN TWO CYPRINIDS FISH: *Barbonymus schwanenfeldii* AND *B. gonionotus* USING SEQUENCING OF CYTOCHROME C OXIDASE I (COI) MITOCHONDRIAL DNA FRAGMENT

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A study was undertaken to examine the phylogenetic relationship between two cyprinids fish (*Barbonymus schwanenfeldii* and *B. gonionotus*) using partial sequencing of cytochrome c oxidase I (COI) mitochondrial gene. Fish samples were collected from two sites in Peninsular Malaysia (36 samples from Serting River and one sample from Pahang River) and two sites in Sarawak (4 samples from Rejang River and 3 samples from Layan River) and one site from Sabah (5 samples from Kinabatangan River). Restriction fragment length polymorphisms (RFLPs) analysis identified four restriction sites (*AluI*, *Csp6I*, *BsuRI*, and *PvuII*) diagnostic between *B. schwanenfeldii* and *B. gonionotus*. Phylogenetic analysis using Neighbor-joining (NJ) method showed that *B. schwanenfeldii* and *B. gonionotus* are reciprocally monophyletic, thus supporting their taxonomic status as distinct species. Additionally, the clustering of mtDNA haplotypes in both species into Peninsular Malaysia and Borneo clades (geographical structuring) possibly resulted from genetic drift related to the separation of Borneo from Peninsular Malaysia during the late Pleistocene epoch.

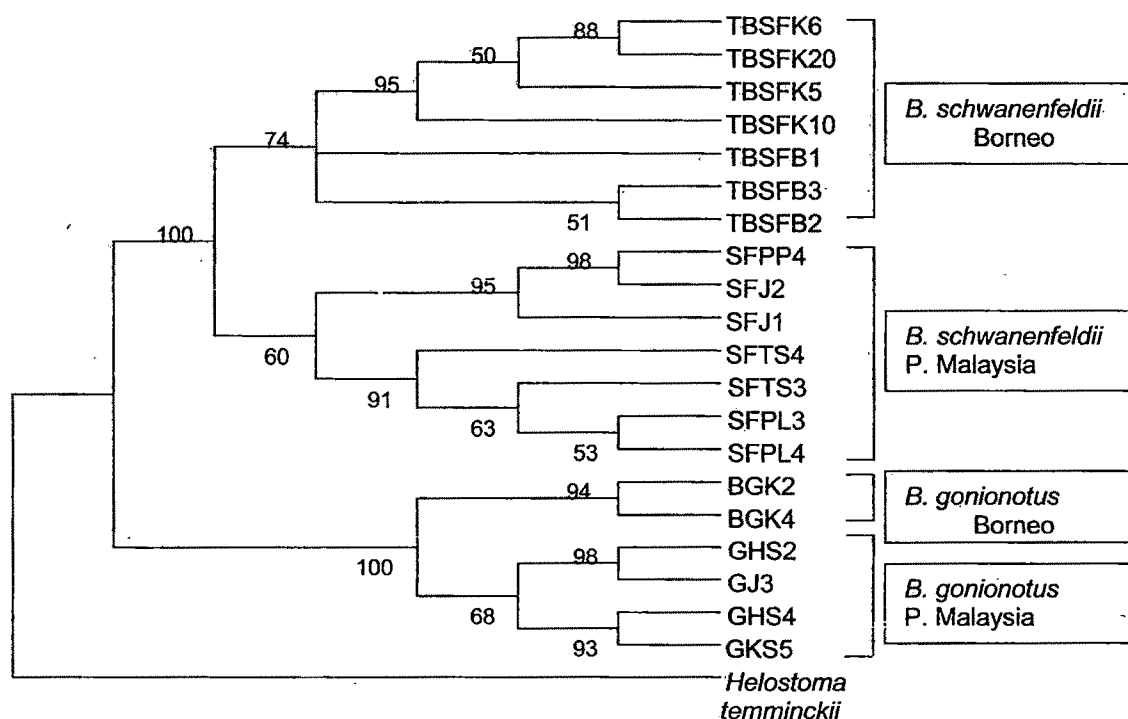


Figure 1 Phylogenetic tree inferred using Neighbour-joining (NJ) method with 1000 replicates. *Helostoma temminckii* was used as outgroup. Abbreviations: BGK1, BGK2 = *B. gonionotus* Borneo; GJ3, GHS2, GHS4 and GKS5 = *B. gonionotus* Peninsular; T-BSFB1, T-BSFB2, T-BSFB3, T-BSFK5, T-BSFK6, T-BSFK10 and T-BSFK20 = *B. schwanenfeldii* Borneo; SFJ1, SFJ2, SFPL3, SFPL4, SFTS3, SFTS4 and SFPP4 = *B. schwanenfeldii* Peninsular Malaysia.

DEVELOPMENT OF TRANSGENIC TECHNOLOGY FOR JAPANESE FLOUNDER

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Aquaculture is becoming widely practiced all over the world, and the level of production has been increasing. However, infectious diseases cause serious problems in the aquaculture industry. Transgenic technology is able to produce new strains of aquaculture species that have important traits more rapidly and easily in comparison with usual breeding techniques. However, fish species targeted for transgenesis have been limited. And, the technique has not been established for aquaculture species. Japanese flounder, *Paralichthys olivaceus* is one of the important culture fish in Japan. For the establishment of a technique for transgenic Japanese flounder, we (1) explored useful promoters derived from Japanese flounder, (2) developed a method for gene transfer into Japanese flounder eggs and (3) constructed an expression vector for producing disease resistance fish strains, and examined it in zebrafish.

5' flanking regions of 4 different genes, gelatinase B, complement component C3, tumor necrosis factor and keratin derived from Japanese flounder were identified and linked to a reporter gene. These promoters showed different regulation manner of gene expression *in vitro* and *in vivo*. These results demonstrated the availability of different promoters for transgenic Japanese flounder depending on various situations.

We tried to establish an efficient gene transfer method for Japanese flounder eggs. The particle gun method is optimal for Japanese flounder transgenesis. The particle gun method produced up to 100 embryos expressing reporter gene in one of the experiments.

We generated transgenic zebrafish that expressed chicken lysozyme gene under the control of Japanese flounder keratin gene promoter. This transgenic strain showed resistance against *Flexibacter columnaris* and *Edwardsiella tarda* infections.

Accumulation of these techniques developed in this study will help realize the production transgenic Japanese flounder, and should contribute to a better understanding of the biology and aquaculture.

**STUDIES ON GENETIC DIVERSITY AND PHYLOGENY OF CHINESE UNIONIDAE
(MOLLUSCA : BIVALVIA)**

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Unionidae is an important group of freshwater benthos. Due to the convergence phenomenon of freshwater mussels, there is still much controversy regarding the classification of Chinese Unionidae. In China, studies of freshwater mussels were mostly on their resources, biology and morphology, while few in the field of genetics, and deficient in their population genetic structure and genetic diversity. Due to increasing contamination of the inland waters such as lakes and rivers, disturbance of habitats and overexploitation of resources of freshwater mussels in recent years, genetic diversity of mussels was damaged to some extent and natural resources of some economic important mussels are on the decline or on the verge of extinction. In order to further understand the status of genetic diversity of different species, it is essential to investigate the molecular phylogenetic relationship of Unionidae on the basis of molecular genetics.

Therefore, RAPD markers were used to detect genetic diversity of twenty populations of eighteen species from ten genera in Chinese Unionidae. Its phylogeny was studied employing the combined analyses of RAPD markers, rDNA ITS1 sequence analysis and shell morphometrics. Promising results have been achieved.

PROTEOMICS ANALYSIS OF HARUAN *Channa striatus* TISSUE EXTRACT

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The snakehead fish *Channa striatus* (*C. Striatus*) locally known as Haruan (in Malay) is an obligatory air-breather and predaceous fish. The fish resides in the slow-flowing streams and crevices near riverbanks in Southern China. Besides being consumed as a dietary protein source, *C. striatus* is used for its medicinal properties, the fish was reported to enhance dermal wound healing and reduce post-operative pain and discomfort.

In our present study, the proteins present in the fish tissue was analysed to evaluate the. Variation in protein expression in three batches of fish samples from three different catches, caught in different months and places in Penang. The fish tissues were first homogenized into a powder form followed by the addition of protein extraction buffer and vortexed. The mixture was then spun at 12000 g for 30 minutes. The supernatant was recovered and subjected to SDS-PAGE protein separation. The gel was then stained with Coomassie Blue.

Twenty-five distinct bands were observed and there were no variations in the protein profiles of the fish samples that were obtained from three different catches. However, when the protein profiles of the fishes were compared according the fish's size, the intensity of the protein bands for the smaller sized fishes (range from 16 cm to 25 cm) were significantly more intense than those of the bigger sized fishes (range from 28 to 38 cm). Thus indicating that with equal dried weight of tissues, the smaller fishes yielded higher amount of proteins than the bigger fishes.

The protein bands were excised and in-gel digestion with trypsin was performed. The resulting tryptic-fragments were subjected to LC/MS/MS analysis and the MS/MS data that contained the amino acid sequence of the protein were used to conduct protein database search. A total of 84 proteins were identified and most of the proteins were kinase enzymes.

GENOMIC APPROACH IN THE STUDY OF ANTIBACTERIAL SEAWEED AND SEA CUCUMBER EXTRACTS INHIBITING GRAM POSITIVE BACTERIAL PATHOGENS

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The utilization of some species of seaweed and sea cucumber extracts for biomedical purposes has been widely known and accepted. However, the species commercially utilized has also been overexploited, making the search for new valuable species an important priority in the research agenda. In addition, sustainable production of the the bioactive products must also be ensured. Harvesting of the whole plant or animals has been the conventional method for extracting the extracts and the biochemical or molecular based activity of the extracts has not been documented.

Two genomic approaches to hasten the search bioactive molecules and understanding molecular based mechanism was utilized in the project that include screening of antibacterial peptides seaweed and sea cucumber as well as the study on nucleotide sequences of *S. aureus* inhibited by the extract. Molecular screening of antibacterial peptides indirectly can pave the way for sustainable supply of bioactive products. The nucleotide changes of selected *S. aureus* genes imply potential drug target sites.

The findings of the research include successful amplification of the gene encoding a peptide with antibacterial ability whereby the the gene encoding for a membrane permeabilizing gene was amplified from a species of sea cucumber tissue which is not the commercialized species. The methanol extract showed selective antibacterial activity whereby only with gram positive pathogens are inhibited. The disc diffusion assay showed the extract exhibited zones of inhibition when grown in plates of gram positive pathogens. The extract inhibited drug resistant and non-drug resistant pathogens.

Another aspect of genomics utilized is the analysis of nucleotide sequence of cell wall protein gene and the DNA repair gene of gram positive bacteria inhibited by red seaweed methanol extract. Nucleotide changes were seen in the DNA repair gene but not the cell wall protein gene of gram positive pathogen upon treatment with the seaweed extract. The disc diffusion assay showed zones of inhibition around the extract when grown in gram positive pathogens but not the gram negative pathogens. Based on these preliminary results, utilizing genomics for screening of biomolecules in natural resources has the potential of speeding up drug discovery programme and the study on nucleotide sequences of treated pathogen can aim at utilizing the biomolecules to target the affected nucleotides so as to inhibit growth of the organism.

THE SV40 T ANTIGEN NUCLEAR LOCALIZATION SEQUENCE (NLS) ENHANCES NUCLEAR IMPORT OF VECTOR DNA IN EMBRYOS OF A CRUSTACEAN, *Litopenaeus schmitti*

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A genetic transformation system for penaeid shrimp could provide a powerful technique for improvement of different production traits of importance for a sustainable aquaculture. The development of a successful transformation system depends upon the ability to efficiently introduce exogenous DNA to the target species. The nuclear localization signal (NLS) peptide of the SV40 T antigen ability to facilitate nuclear import and transient gene expression of transgenes is known from vertebrate systems and is here for the first time has been shown in a crustacean using embryo from shrimp *Litopenaeus schmitti*. Electroporation was used to introduce pCMV-*lacZ*; a plasmid that contains the human cytomegalovirus promoter/enhancer (CMV) fused to the β -galactosidase (*lacZ*) coding region into *Litopenaeus schmitti* zygotes. Supercoiled DNA was used at 50 or 500 ng/ μ l with or without associated NLS peptides. The hatching frequency of electroporated zygotes was around 60% for all groups apart from the pCMV-*lacZ*: NLS group at 500 ng/ μ l (43%). Based on Southern blot analysis of PCR products the gene transfer frequency was two-fold higher using DNA: NLS complexes than with naked DNA (23.8% vs. 11.5%, with 50 ng/ μ l of plasmid DNA; 44.3% vs. 28.8% with 500 ng/ μ l). The β -galactosidase assay indicated that nuclear uptake is faster for the DNA: NLS complexes than for naked DNA. The β -galactosidase activity was always higher in the DNA: NLS groups than in the naked DNA groups. To our knowledge, this is the first report of the use of a NLS peptide to improve gene transfer and nuclear uptake in penaeid crustaceans.

GENETIC VARIABILITY OF THE MARBLE GOBY *Oxyeleotris marmoratus* BASED ON THE PARTIAL REGION OF THE MITOCHONDRIAL CYTOCHROME B GENE

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In this study, we analysed sequence variation in PCR amplified partial fragment of cytochrome *b* gene of 25 individuals of the marble goby, *Oxyeleotris marmoratus* collected from five populations in Peninsular Malaysia. A 350 bp fragment of mitochondrial cyt *b* gene was amplified by using universal primer. Interpopulation genetic distance values were calculated among the five populations based on Kimura 2 parameter. Genetic distance and maximum parsimony analyses as well as phylogenetic tree construction were done using PAUP 4.0b1. Outgroup sequence were obtained from Genbank. The dendrogram produced showed two major clusters. The first cluster consisted of four individuals from Kelantan and the outgroup. The rest formed another major cluster which divided into four sub-clusters. The first sub-cluster contained all individuals from Johor and another four individuals from Kelantan. The second sub-cluster gave two nodes, first node consisted of all individuals from Pulau Keladi, Pahang while three individuals from Tasik Banding, Perak, formed the other node. The other sub-clusters consisted of individuals from Tasik Banding, Perak. Genetic distances ranged from 0.0037 – 0.0598. Bootstrap support values ranged from 52% to 88%. The sequence comparisons of different geographic populations give estimates of their genetic relatedness and provide genetic information which could be applied for further genetic improvement in a breeding programme.

CULTURE OF TIGER SHRIMP *Penaeus monodon* IN A ZERO WATER EXCHANGE SYSTEM

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A major issue for the shrimp farming industry is the environmental impact of discharged nutrients from shrimp farms into natural water sources. The effluent has the potential to increase algal blooms, deplete oxygen at the bottom of water bodies and reduce biodiversity. Most of the discharged nutrients originate from unused feed and faeces. Disease outbreaks are intensified by poor water quality and high density of farms sharing water intakes and outlets. One approach to improve farming sustainability and biosecurity is development of high-intensity grow out systems with no water discharge over the crop cycle. This study evaluated a closed cultivation system for viability and profitability of tiger shrimp production while reducing discharge of nutrients and suspended solids into the environment. The treated pond used a locally available bioremediation set while the control pond was operated using normal water exchange procedures. The culture period was within 120 days. Both control and treated ponds had good survival (72.4% and 71%, respectively), mean final weights were 15.5 and 17.3 g, respectively, and FCR were 2.0 and 1.8, respectively. In addition, no significant differences were observed between the control and treatment pond for any of the daily water quality parameters measured (dissolved oxygen, pH, temperature, salinity and Secchi disk readings) or any of the weekly water quality parameters measured (total ammonia-N, nitrite-N, nitrate-N, reactive-P, total-P, BOD and TSS).

THE GROWTH PERFORMANCE OF JAPANESE ABALONE, *Haliotis discus hannai* IN THE RECIRCULATING AQUACULTURE SYSTEM

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Abalone culture is expanding in several countries in response to decline in production from fisheries. Recirculating aquaculture systems (RAS) reduce many of the obstacles related to predation, weather conditions, and effect of waste water, allowing consistent quality of product and environmental friendly aquaculture. This research was conducted to evaluate the growth performance of Japanese abalone, *Haliotis discus hannai* in the RAS. The experiment was performed for juvenile abalone with shell length of 24.5 mm at temperatures of 16°C in the RAS. Abalones were divided into three groups with stocking densities, 700 (Group A), 1,300 (Group B), 1,910 (Group C) individuals/m² bottom area of culture tank and were randomly stocked in triplicate. The system with water volume of 7 m³ consisted of 9 tanks, a fluidized sand filter, a protein skimmer and two hydrocyclones.

Table 1 The growth performance of Japanese abalone, *Haliotis discus hannai* in the recirculating aquaculture system for 125 days (P<0.05)

		A group	B group	C group
Initial	Total (g)	1,549.3±40.02 ^c	2,962.0±17.44 ^b	4,103.0±53.69 ^a
	NO.	700	1,300	1,910
	shell length (mm)	24.72±0.19 ^a	24.52±0.24 ^a	24.22±0.23 ^a
Final	Total (g)	2,213.8±12.93 ^c	4,062.5±48.33 ^b	5,610.4±50.08 ^a
	NO.	646	1,185	1,673
	shell length (mm)	34.27±0.49 ^a	33.43±0.96 ^a	32.89±0.50 ^a
Growth	Daily feeding rate (%)	8.2±0.41 ^a	7.9±0.21 ^a	7.7±0.16 ^a
	Daily growth rate (%)	0.28±0.28 ^a	0.26±0.01 ^b	0.25±0.00 ^b
	Daily increase of the shell length (µm/day)	76.5±2.39 ^a	71.2±5.79 ^a	69.4±2.10 ^a
	Feed Conversion	24.7±0.3 ^b	25.7±0.0 ^a	25.7±0.0 ^a
	Survival rate (%)	93.2±1.1 ^a	91.9±1.1 ^a	88.6±0.5 ^b

EFFECT OF PARTIAL OR TOTAL REPLACEMENT OF FISH MEAL PROTEIN BY SOYBEAN MEAL PROTEIN ON GROWTH, FOOD UTILIZATION AND PANCREATIC ENZYME ACTIVITIES IN AN INDIAN MAJOR CARP *Labeo rohita*

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This study was undertaken to determine whether the partial or total replacement of fish meal protein by protein from solvent-extracted, commercially available soybean meal would provoke a response in pancreatic proteolytic enzyme secretion in *Labeo rohita* fingerlings. At the end of the growth trial (60 days) the activity of chymotrypsin was determined by an *in vitro* method based on the oral introduction of N-benzoyl-L-tyrosyl-p-aminibenzoic acid (bt-PABA). Comparative trials were run with fish forced fed with PABA. The extraction index (bt-PABA/PABA) reflected the decreasing rate of pancreatic chymotrypsin activity with increasing amount of soybean protease inhibitors introduced into diet. This finding is in contrast to the several fold increase of trypsin activity in faeces collected from fish fed a soybean meal supplemented diet. The growth rate of fish was reduced significantly when 50% of the fish meal was replaced by soybean meal, and 100% replacement resulted in growth arrestment and mortality. It is suggested that the increase in secretion of pancreatic proteases caused by the soybean protease inhibitors does not compensate for the decrease in the efficiency of dietary protein digestion.

**USED OF MICROBIAL PHYTASE REPLACE INORGANIC PHOSPHORUS IN SEX-
REVERSED RED TILAPIA *Oreochromis nilotic us* (LINNAEUS) X *Oreochromis
mossambicus* (PETERS)**

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The effects of a microbial phytase (liquid Ronozyme P) derived from *Aspergillus niger* (Roche, Basel, Switzerland) on digestibility, and utilization of phosphorus (P) originating from plant materials were studied in sex-reversed red tilapia. Diets were mainly composed of soybean meal and contained P only from plant materials. The basal diet (Diet 1) was formulated to contain 30% protein, 3.5 kcal digestible energy/g, 0.78% total phosphorus, and 0.26% available phosphorus. Microbial phytase was added to the basal diet at 0, 500, 1,000, 2,000 and 4,000 units/kg, respectively (Diets 1 to 5). Diets 6 and 7 were the same as Diet 1 except that 0.2 and 0.3% feed grade dicalcium phosphate were added (calculated total available phosphorus levels in the diets were 0.46 and 0.56% respectively). Five replicate groups of fish (20 tilapia/tank) of mean initial weight of 5.5 g were stocked into 235-l flow-through aquaria for 10 wk. The results indicated that the supplementation of microbial phytase 1,000 units/kg provided the best phosphorus digestibility coefficient which was sufficient for tilapia feed. An increasing trend was noted for hematocrit and hemoglobin in tilapia fed the diets containing 1,000 units of microbial phytase/kg and above. In addition, serum phosphorus markedly increased when tilapia was given diets with over 1,000 units of microbial phytase/kg while the supplementation of 500 units of microbial phytase/kg increased the serum Zn. Higher levels of phosphorus was retained in the bone and the lower level of phosphorus present in the feces of tilapia fed the diets containing phytase resulted in less phosphorus being excreted into the pond water.

APPARENT PROTEIN DIGESTIBILITY OF *Gliricidia sepium* J. LEAF MEAL (GSLM) BY MOZAMBIQUE TILAPIA *Oreochromis mossambicus*

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Digestibility of feedstuffs is one of the most important aspects in the evaluation of the biological as well as economic effectiveness of any feedstuffs. Generally, it is a well established fact that feedstuffs of plant origin are less digestible to fish than those from animal origin (NRC, 1983). This is due, mainly, to the relatively high fiber content in plant materials than feedstuffs from other sources. There are no previous reports on the possible use of *G. sepium* leaf meal (GSLM) in fish diet. The objective of this study was therefore to investigate the apparent protein digestibility if GSLM by Mozambique Tilapia, *O. mossambicus*.

The apparent protein digestibility of *Gliricidia sepium* J. by African cichlid (*O. mossambicus*) was determined. The conventional indirect method of digestibility determination has been used in this study. Accordingly, one percent chromic oxide was added to the total *G. sepium* leaf meal (GSLM) diet and the chromic oxide concentration both in the diet and feces was analyzed following the acid digestion method. The result obtained has prevailed that *O. mossambicus* digested about 56% of protein in GSLM. It is concluded therefore that *G. sepium* is considered fairly digestible by Tilapia (*O. mossambicus*) and is quite better than the digestibility of similar fodder legume leaf meals (Table 1).

Table 1 Protein digestibility (%) of *G. sepium* leaf meal (GSLM) compared with the digestibility of similar feed ingredients

Feedstuff	Digestibility (percent)
GSLM-(this experiment)	56.0
Fishmeal*	84.4
Soybean meal*	94.4
Leucaena leaf meal (soaked)**	41.0
Leucaena leaf meal (sun-dried) **	40.0
Alfalfa meal*	65.7
Coffee pulp*	29.2

* Lovell (1989).

** Wee & Wang, 1987.

UTILIZATION OF BREWERY WASTE IN SUPPLEMENTARY DIET FOR INDIAN MAJOR CARPS: EFFECT ON FLESH QUALITY

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Brewery waste includes brewer's spent grains left after extraction of malt required for the production of beer. One hundred kilogram of barley produces 170 kg of wet brewer's grains (having 80-85% moisture). The brewery waste contains 19% crude protein, 3-5% ether extract, 18-20% crude fiber and a good profile of amino acids (NRC, 1983).

The present studies were conducted to observe the suitability of brewery waste as a feed ingredient of carp diet and also to find out the appropriate level of its inclusion in the diet. The brewery waste was used at four different levels (10%, 20%, 30% and 40%), replacing rice bran (at 25%, 50%, 75% and 100% levels) in traditional diet, composed of rice bran (40%), mustard deoiled cake (40%) and fish meal (20%), of Indian major carps, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. Brewery waste (spent brewer's grains) was collected from M/s 'United Brewers' Ludhiana. In fresh form, it contained 80-85% moisture, 24.5% crude protein, 6.90% ether extract, 20.5% crude fiber, 40.6% nitrogen free extract and 4.7% total ash. The waste was sun dried for 3-4 days and then oven dried at 70-80°C for 12 hours to make it completely moisture free. Experiments were conducted for 12 months in outdoor cemented tanks.

Results in terms of net weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio showed that brewery waste can be utilized at 30% level in the diet of *L. rohita* and *C. catla*, but in *C. mrigala* it does not showed positive results. Flesh quality was determined at the end of the experimental period. Maximum flesh protein content in all the three fish species was recorded in the fish fed on diet having 40% brewery waste, while lowest in fish fed on diet having 10% brewery waste. On the contrary, the flesh lipid content showed a reverse trend i.e. maximum lipid content were observed in the diet without brewery waste in all the three fish species, while lowest flesh lipid were recorded at 40% level. The maximum total sugar content of flesh in *C. catla* and *C. mrigala* were recorded at 30% inclusion level of brewery waste, while in *L. rohita* in diet without brewery waste. The maximum moisture content were recorded at 10% inclusion level of the brewery waste in all the three fish species, while minimum at 30% inclusion level of brewery waste in *C. catla*, in diet without brewery waste for *L. rohita* and at 40% in *C. mrigala*. The maximum ash content were recorded at 10% inclusion level of brewery waste in *C. catla*, while in diet without brewery waste for *L. rohita* and *C. mrigala*. Results clearly revealed that increase in fish biomass with inclusion of brewery waste was not due to the increase in water content in flesh

In terms of cost of diets, diet having 30% brewery waste costs about Rs. 5.15/kg, in comparison to control diet, which costs Rs. 6.20/kg.

The better growth performance and flesh quality of carps fed on diets containing brewery waste has been attributed to the good quality of the waste as compared to rice bran (crude protein 12.87%, ether extract 1.50%, total ash 11.18%), availability of good quality protein as the brewery waste contains essential amino acids like lysine, arginine and methionine (William, 1955) comparable to fish meal and about thrice the level of these amino acids in rice bran (Lovell, 1989).

**THE INCLUSION OF UREA-TREATED PALM KERNEL MEAL AS A FEED INGREDIENT
IN THE DIET OF JUVENILE SEABASS *LATES CALCARIFER* AFFECTS GROWTH
PERFORMANCE**

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A 56-day feeding trial was conducted to evaluate the effect of using palm kernel meal (PKM) and chemically-treated PKM using urea (UTPKM) as feed ingredients in diets on the growth performance of juvenile seabass *Lates calcarifer*. Seven isonitrogenous (45% crude protein), isoenergetic (19 kJ g⁻¹) and isolipidic (10% crude lipid) practical experimental diets were formulated. The control diet (Diet 1) did not contain any PKM and the other six diets were formulated to have either raw PKM or UTPKM at 7.5 %, 15.0 % or 22.5 % of the diet respectively, at the expense of solvent-extracted soybean meal. Danish fish meal was included at 39.7% for all diets so that at least 60% of the total dietary protein came from animal protein. Growth performance of seabass juveniles with initial weight of 49.8 ± 0.2g (mean ± s.d.) fed the control diet, 7.5% PKM and 15% PKM or 7.5% UTPKM, 15% UTPKM and 22.5% UTPKM diets showed similar growth ($P > 0.05$) and better performance than fish fed the 22.5% PKM diet ($P < 0.05$). Mean final weights of seabass fed all diets except 22.5% PKM diet were not significantly different ($P > 0.05$) compared to seabass fed with the control diet. Similar trends were observed in terms of percent weight gain and specific growth rates. On the other hand, in terms of protein efficiency ratio (PER) and net protein utilization (NPU), there were significant differences ($P < 0.05$) among all dietary treatments with the control diet giving the highest PER and NPU of fish compared to fish fed 15.0% PKM or 22.5% PKM. Nevertheless, the PER and NPU of fish fed with either 7.5% PKM, 7.5% UTPKM or 15% UTPKM showed similar performance with the control diet ($P > 0.05$). It was concluded that PKM is a potential partial replacement for soybean meal in the diets of juvenile seabass and that urea treatment of PKM may further improve the utilization of this by-product from the oil palm industry.

EFFECTS OF USING (N-3) HUFA- ENRICHED *Artemia* AS STARTER FOOD ON GROWTH AND SURVIVAL OF RAINBOW TROUT *Oncorhynchus mykiss* LARVAE

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In this experiment, the effects of using newly hatched *Artemia* and enriched *Artemia* as starter food on growth and survival of rainbow trout larvae were investigated and compared with artificial food. Larvae with absorbed yolk sack with weight of 92.2 mg were reared for 20 days using four different food types: (1)- Artificial food, (2)- Newly hatched *Artemia*, (3)- Enriched *Artemia* and (4)- 50 % artificial food and 50 % enriched *Artemia*. Triplicates were used for each treatment. At the end of experiment, highest percentage of weight gain (104.4 ± 9.47) was observed in treatment 3 ($P < 0.05$) and highest survival ($96.5 \pm 1.69\%$) occurred with larvae fed treatment 4 ($P < 0.05$). In view of body composition, larvae fed with enriched *Artemia* had highest levels of unsaturated fatty acids (n-3) compared to larvae fed other dietary treatments.

EFFECTS OF DIETARY CASSAVA LEAF AND ROOT MEAL ON GROWTH PERFORMANCE AND BODY COMPOSITION OF AFRICAN CATFISH *Clarias gariepinus*

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An experiment was carried out to evaluate the effect of different levels of cassava leaf meal (CLM) and cassava root meal (CRM) in the diets of *Clarias gariepinus* as a partial replacement of soybean meal. The total cyanide content of oven dried cassava root meal and cassava leaves were 13.96 ppm and 56.28 ppm respectively. Nine isonitrogenous and isocaloric diets were formulated with two different levels of cassava leaf meal alone and in combination with three levels of cassava root meal as follows: diet 1 as a control diet (containing fish meal and soybean meal), diet 2 (10 % CLM alone), diet 3 (10 % CLM and 10 % CRM), diet 4 (10 % CLM and 20 % CRM), diet 5 (10 % CLM, 30% CRM), diet 6 (20 % CLM alone), diet 7 (20 % CLM, 10 % CRM), diet 8 (20 % CLM and 30 % CRM), diet 9 (20 % CLM and 30 % CRM). Triplicate groups of *Clarias gariepinus* with initial body weight of 1.42 ± 0.11 g were hand fed twice at 7 %/ body weight/ day for 12 weeks. Growth performance, feed utilization and nutrient digestibility were studied.

The results showed that growth performance and feed utilization efficiency were significantly better in the fish fed control diet with the best FCR (1.74 ± 0.05) and SGR (3.22 ± 0.04) than those fish fed the cassava based diets. The highest apparent protein digestibility (83.11 ± 1.17) and total digestibility (52.56 ± 0.24) were also obtained in the control diet. This was followed by fish fed diet containing 10 % cassava leaf meal alone with a higher FCR (2.013 ± 0.04) and SGR (3.03 ± 0.03) compared with the diet containing 20 % of cassava leaf meal alone. There was a trend of reduced growth and feed utilization when increasing levels of cassava root meal was incorporated in the cassava leaf meal based diets, but the differences were not significant among the diets. Inclusion of 30 % cassava root meal gave the poorest FCR (3.28 ± 0.1) and the least SGR (1.89 ± 0.04). Diets containing cassava could cause an imbalance of amino acid profile especially methionine content, lower digestibility and palatability. The data obtained in this study indicate that a diet containing up to 10 % of cassava leaf is acceptable and could be used for least-cost formulation in the catfish diet. However, to include the cassava root, the maximum level is suggested not exceeded 20 % inclusion.

THE PROTEIN REQUIREMENT OF SILVER BARB *Barbodes gonionotus* LARVAE

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Silver barb is one of the most important aquaculture species in Southeast Asia. High cost of live food used at the larval stage hampered the culture development of this species. This study was conducted to determine the protein requirement of silver barb. Five isocaloric diets (18.4 kJ g⁻¹) containing 40-60% crude protein were formulated in microbound form. Five-day old larvae (3.38 mm and 0.08 mg) were obtained from artificially spawned brooders using carp pituitary extract. Larvae were fed to satiation thrice a day for 16 days. Growth, survival and body composition of the larvae were determined at the end of the experiment.

Growth increased as the protein level increased up to a maximum level of 55% and decreased as the protein level increased further to 60%. Survival of the larvae was highest when they were fed 50% dietary protein but this was not significantly different from those larvae given 55% protein. Body protein increased when the dietary protein was increased with the exception of larvae fed on the 50% protein diet. Body lipid and ash contents of the larvae were however, not significantly different among dietary treatments.

Table 1 Survival (%), growth performance and feed efficiency of silver barb larvae fed the experimental diets with varying protein levels for 16 days¹

Diet	Survival	Body weight		Total Length	
		Final (mg)	Gain (%)	Final (mm)	Gain (%)
40	55.7±9.4 ^b	8.0±1.3 ^c	843.7 ± 53.1 ^c	7.7±0.3 ^c	130.3 ± 9.3 ^c
45	55.0±3.5 ^b	9.8±0.1 ^{bc}	1054.0 ± 21.0 ^{bc}	8.2±0.0 ^c	142.8 ± 1.1 ^{bc}
50	71.1±3.1 ^a	12.2±1.7 ^{ab}	1342.4 ± 380.0 ^{ab}	9.2±0.3 ^b	172.2 ± 31.0 ^b
55	62.8±3.1 ^{ab}	13.6±3.1 ^a	1506.6 ± 194.0 ^a	10.8±0.5 ^a	213.6 ± 53.5 ^a
60	42.4±2.8 ^c	10.1±0.7 ^{bc}	1089.0 ± 92.6 ^{bc}	8.5±0.2 ^{bc}	144.57 ± 4.7 ^{bc}

Table 2 Body composition (% ± SEM) of silver barb larvae fed diets with varying dietary protein levels

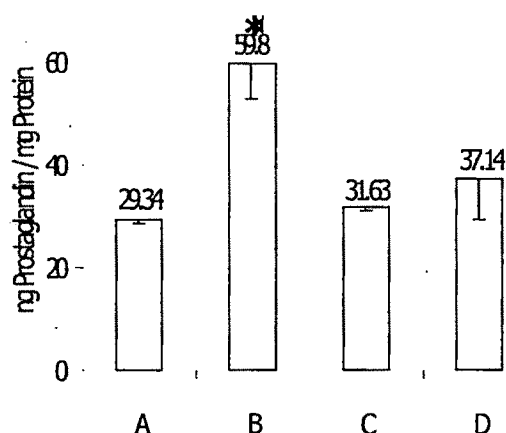
DIET	PROTEIN	LIPID	ASH
40	56.3 ± 0.7 ^{BC}	15.5 ± 1.3 ^A	11.1 ± 1.9 ^A
45	56.9 ± 2.1 ^{BC}	12.2 ± 0.8 ^B	13.8 ± 2.4 ^A
50	54.8 ± 3.0 ^C	14.7 ± 1.3 ^{AB}	9.6 ± 1.5 ^A
55	59.7 ± 1.4 ^{AB}	14.1 ± 1.6 ^{AB}	12.1 ± 2.8 ^A
60	62.1 ± 1.8 ^A	14.3 ± 1.9 ^{AB}	11.3 ± 2.8 ^A

IDENTIFICATION OF REPRODUCTIVE HORMONES IN POLYCHAETES *Perinereis* sp. AND *Marphysa* sp.

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Polychaetes have long been acknowledged by aquaculturists to be the most suitable for marine prawn maturational diets over other natural feeds owing to their ability to enhance reproductive performance of prawns. Nevertheless, there is almost no research for identifying such critical maturational enhancing factors in polychaetes. Based on knowledge that prawn reproductive system is influenced by hormonal and neuronal factors, it is possible that hormonal contents of polychaetes might be one of the reasons for the maturation of prawns. We prepared polychaete extracts to identify the existence of some reproductive hormones such as vertebrate-type steroid hormones and eicosanoids by radioimmunoassay, enzyme-immunoassay, radiochemical assay and high performance liquid chromatography. The results showed that polychaetes contained progesterone, prostaglandin and a terpenoid hormone-like substance. In an attempt to clarify the effects of each hormone on ovarian development, immature ovaries of freshwater prawn were incubated with progesterone and prostaglandin *in vitro* at concentrations of 500 pg/ml and 1000 pg/ml. Percentages of mature oocytes increased in hormones treatments.

Figure 1 Prostaglandin levels (ng/ mg Protein) in whole homogenate of polychaetes analysed by enzyme immunoassay (average $\pm$ sd). Data with asterisk indicates highly significant number ($p < 0.05$).



A = 6 months old *Perinereis* sp.
B = 8 months old *Perinereis* sp.
C = 6 months old *Marphysa* sp.
D = 8 months old *Marphysa* sp.

THE AVAILABILITY OF AN OPTIMAL DIET AS AN IMPERATIVE FACTOR ON THE DOMESTICATION OF TIGER SHRIMP *Penaeus monodon* IN CONCRETE RACEWAY

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Present supply of tiger shrimp spawners come from the wild and these spawners are found to be carriers of certain diseases. For this reason, there is a need to develop domesticated or farm-raised tiger shrimp broodstock in view of the fact that 80% of natural broodstocks generally died during capture as a consequence of handling difficulty. Of those that survive, only about 30% were not white spot syndrome virus carriers with only 6% would be spawners. Domesticated broodstock could ensure more reliable supply of specific pathogen free postlarvae, which is a major requirement for sustainable tiger shrimp farming. One of the needs in farm-raised tiger shrimp to reach broodstock size is optimal diet for better growth and higher sexual maturity rate. Fresh feed such as squid and fish fillet could enhance the growth and survival rate of the shrimp.

The objective of this study was to identify the optimal diet for better growth and survival rate of farm-raised tiger shrimp broodstock. Marketable sized tiger shrimp weighing approximately 38g each were obtained from a local farm and stocked in three concrete raceways sized 4m x 40m with a stocking density of 5 shrimp/m². Shrimp in tank A, the control tank, were fed with pelletised feed (monodon feed; Grobest no.5), shrimp in tank B were fed with fresh food (squid and fish fillet) and shrimp in tank C were fed with artificial-homemade feed (combination of pelletised feed with fresh feed). The study showed that growth and survival rate of shrimp that was fed with artificial-homemade feed was significantly higher than that which were fed with the commercial pelleted feed and fresh feed.

AN IMBALANCE OF EAA/NEAA AND NOT THE ANTAGONISM OF LYS-ARG AFFECTS GROWTH AND FEED UTILIZATION OF YELLOW *Mystus nemurus*

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Effects of an excess or imbalance of amino acids on growth and metabolism of nutrients in yellow mystus is scarce. The aim of this study was to investigate effects of an excess of either lysine or arginine on growth and feed utilization of juvenile yellow mystus. Diet 1, the control diet contained fish meal, gelatin and crystalline essential amino acids (EAA) simulating the amino acid profile of *Mystus nemurus* except for lysine and arginine which were incorporated at the required levels of 3.46 and 4.80 % of protein, respectively. Diets 2 and 3 were formulated to contain an excess level of lysine and arginine, respectively. Diet 4 contained both lysine and arginine at an excess level. Catfish with average initial weights of 6.75-6.82 g were fed their respective experimental diets for 8 weeks.

Fish fed diet 1 had the poorest specific growth rate (SGR), feed conversion efficiency (FE), protein efficiency ratio (PER), and productive protein value (PPV). Growth and feed utilization of fish fed diets 2, 3 and 4 were normal and significantly higher than those of fish fed diet 1. Although diet 1 contained lysine and arginine at the required levels, the amount of non-essential amino acids (NEAA) was very high making up 66.6% of total crystalline amino acids. NEAA levels in diets 2, 3 and 4 were 51.51, 49.44 and 39.22% of total crystalline amino acids, respectively. Though not significantly different, fish fed diets 2 or 3 had better feed intake and specific growth rate. The results indicated that an imbalance of EAA/NEAA in the diet had an effect on growth and feed utilization of yellow mystus and that the antagonism of lysine-arginine was not observed in the present study.

Table 1 Feed intake, final weight, specific growth rate and feed utilization of yellow mystus fed diets containing excess lysine and arginine levels

Diet	Feed intake (g/fish)	Final weight (g/fish)	SGR (%)	FE	PER	PPV (%)
1 (3.46, 4.80) ¹	13.16± 0.93	14.39±0.88	1.31±0.19	0.57±0.06	1.63±0.18	24.95±1.73
2 (13.86, 4.80)	23.60± 0.28	24.15±0.71	2.23±0.07	0.74±0.03	2.13±0.07	33.24±1.92
3 (3.46, 15.08)	27.98± 1.83	26.70±1.31	2.41±0.08	0.71±0.02	2.11±0.05	32.07±2.26
4(13.86,15.08)	25.33± 8.73	24.72±8.50	2.19±0.52	0.69±0.09	2.08±0.26	32.58±4.12

¹ Numbers in parenthesis are arginine and lysine levels (% of protein)

DIET COMPOSITION OF THREE CATFISHES FROM AL-HAMMAR MARSH, AL-FUHOUD, IRAQ

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The stomach contents of three catfishes were studied. These were Asian Jieri, *Silurus triostegus* Heckel, 1843, Abu Al-Hakam *Heteropneustes fossilis* (Bloch, 1794), and Abu Al-Zommer *Mystus pelusius* (Solander in Russell, 1794). Fishes were collected from Al-Hammar Marsh near Al-Fuhoud town between March 1989 and February 1990. Small fish formed 70.7% of the *S. triostegus* consumed food by volume and 83.2% according to ranking index. While shrimp and insects larvae were ranked first and formed 47.7% and 59.4% of *M. pelusius* diet according to volumetric method and ranking index, respectively. Detritus and non-nutritional materials comprised 51.7% and 67.9% of *H. fossilis* stomach contents according to the above two methods of analyses respectively. *M. plusius* was the most active feeding fish (86.6%).

AVAILABILITY AND QUALITATIVE STATUS OF COMMON SHRIMP FEEDSTUFFS IN KHULNA DISTRICT OF BANGLADESH

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A survey on shrimp feed markets was carried out in all shrimp farming upazilas (sub-districts) of Khulna district in Southwestern Bangladesh to study the availability, seasonal change of quality and economic use of feedstuffs in shrimp farming. The market survey for availability and price of feedstuffs and collection of feedstuff samples from four selected markets for proximate composition analysis were done at three month intervals from January, 2002 to December, 2003. The results of the study showed that the commonly used feedstuffs were generally available throughout the year in all freshwater prawn (*Macrobrachium* spp.) farming upazilas while it was less available in penaeid shrimp farming areas. The most important feed ingredient fish meal was moderately available only in freshwater prawn farming areas in 2002 but the availability decreased in the following year. The price of commonly available indigenous feed ingredients such as fish meal, mustard oil cake, sesame oil cake, coconut oil cake, rice polish and wheat bran ranged between 17.50-40.00, 8.00-14.00, 8.50-13.00, 7.50-12.50, 5.25-8.00, and 6.80-12.00 Taka (1 US\$ = 62 Bangladesh Taka) per kg respectively. The price of imported feed ingredients like soybean oil cake, meat and bone meals and protein concentrates ranged between 12.00-19.25, 19.00-26.00 and 33.00-40.00 taka per kg respectively. Significant ($P < 0.05$) seasonal variation in price of fish meal and wheat bran was observed while the seasonal variation was not significant ($P > 0.05$) in price of various oil cakes and rice polish during the two seasons study period. Crude protein content of fish meal, mustard oil cake, sesame oil cake, coconut oil cake, rice polish and wheat bran ranged between 25.29-67.37%, 29.74-38.10%, 23.5-33.00%, 14.00-21.00%, 10.50-16.80% and 13.40-19.20% respectively. Nutritive value of locally produced fishmeal generally was of poor quality due to deliberate addition of non-protein waste materials during milling by the miller. No significant ($P > 0.05$) seasonal variation was observed in protein content of feedstuffs during the study period except for fish meal and rice polish where the variation was significant ($P < 0.05$). It was assumed that the qualitative variation of fish meal and rice polish was mainly due to addition of other material and rice husks respectively, not due to the seasonal change. Soybean oil cake, meat & bone meals and protein concentrates were generally of good quality containing 40-50%, 45-55% and 54-66% crude protein respectively. In highlighting economic protein source, mustard oil cake was the cheapest (32.36 Tk./kg protein), followed by soybean oil cake (36.26 Tk./kg protein), meat and bone meals (38.52-50.87 Tk./kg protein), sesame oil cake (41.15 Tk./kg protein) protein concentrates (55.60-66.26 Tk./kg protein) and fish meal (65.61-69.98 Tk./kg protein). The results of the survey indicated that cost-effective compounded pelleted feed might be formulated using these locally available feed ingredients for small-scale commercial shrimp farming.

COMPENSATORY GROWTH OF JUVENILE FLOUNDER *Paralichthys olivaceus* FED COMMERCIAL FEED DURING SUMMER SEASON

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In the occurrence of red tide or coldwater mass, fish are most likely to be starved until it disappears. Fish farmers are suffering from great economical loss due not only to high mortality directly resulting from red tide or coldwater mass, but also from reduction in fish growth due to starvation. The objectives of this study were to investigate possibility of compensatory growth of juvenile flounder fed commercial feed during the summer season. Twenty five juvenile (Mean weight $\pm$ S.D.: 37.9 $\pm$ 0.26 g) fish were stocked and hand-fed commercial sinking feed containing 55.6% crude protein and 8.0% crude lipid. Five treatments of fish with 3 replicates were prepared. Fish in the control group (C) was hand-fed to satiation twice daily during 6 weeks. Fish in the S1, S2, S3 and S4 experienced 1, 2, 3 and 4 weeks of starvation, respectively, before being fed to satiation twice daily during the rest of feeding trial period.

Survival of flounder in C, S1 and S2 was significantly higher than that of fish in S4. Weight gain of flounder in C and S1 was significantly higher than that of fish in S2, S3 or S4. And weight gain of fish in S2 and S3 was significantly higher than that of fish in S4. Feed consumption of flounder linearly decreased with weeks of feeding, except for that of fish in S3. FER and PER for flounder in C, S1, S2 and S3 were significantly higher than those for fish in S4. This study showed that compensatory growth was obtained in juvenile flounder properly fed by the commercial feed for 5 weeks after 1-week feed deprivation during the summer season. Compensatory growth of flounder was well supported by improvement in SGR, FER and PER for flounder in this study.

Table 1 Survival (%), weight gained (g/fish), feed supply (g/fish), feed efficiency ratio (FER) and protein efficiency ratio (PER) of juvenile flounder with different feeding strategies. Means in the same row followed by different superscript letters are significantly different ($P < 0.05$).

Treat.	Final W.T. (g/fish)	Survival (%)	W.T. gained (g/fish)	Feed supply (g/fish)	FER	PER
C	90.7 $\pm$ 2.82a	77.3 $\pm$ 5.81a	52.8 $\pm$ 2.78a	45.2 $\pm$ 1.36a	1.17 $\pm$ 0.026a	2.10 $\pm$ 0.047a
S1	83.1 $\pm$ 6.13a	80.0 $\pm$ 0.00a	45.1 $\pm$ 5.80a	35.7 $\pm$ 4.35b	1.26 $\pm$ 0.009a	2.27 $\pm$ 0.016a
S2	67.9 $\pm$ 4.54b	84.0 $\pm$ 9.24a	30.0 $\pm$ 4.60b	25.6 $\pm$ 2.20c	1.16 $\pm$ 0.089a	2.09 $\pm$ 0.160a
S3	59.0 $\pm$ 0.54b	60.0 $\pm$ 18.04ab	20.9 $\pm$ 0.44b	21.3 $\pm$ 2.59c	1.01 $\pm$ 0.115a	1.82 $\pm$ 0.206a
S4	39.2 $\pm$ 2.95c	22.0 $\pm$ 6.00b	1.3 $\pm$ 2.71c	9.6 $\pm$ 0.28d	0.14 $\pm$ 0.286b	0.25 $\pm$ 0.515b

**EFFECT OF FEEDING FREQUENCY WITH DIFFERENT FEED TYPES ON GROWTH
AND BODY COMPOSITION OF FLOUNDER *Paralichthys olivaceus***

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A 7-week feeding experiment was conducted to determine the proper feeding frequency with different feed types for growth and body composition of the juvenile flounder *Paralichthys olivaceus*. Three replicate groups of fish with average weight of 11.1 g were fed to satiation with moist pellet (MP), sinking dry pellet (DP) and floating extruded pellet (EP) at different feeding frequencies (three, two and one meal a day or one meal every 2 days). At the end of the feeding trial, gastric evacuation rate of the fish was monitored after feeding.

Survival of fish was not affected by either feed type or feeding frequency. Weight gain, feed efficiency, protein efficiency ratio, and daily feed intake of the fish were affected by feed type and feeding frequency. Weight gain and daily feed intake of fish increased with increasing feeding frequency at each feed type. The highest weight gain of fish fed the EP diet three times daily was not significantly different from that of fish fed the DP diet three times daily. Feed intake of fish fed the MP and DP diets showed a higher tendency than that of fish fed the EP diet at each feeding frequency. Feed efficiency and protein efficiency ratio of the fish tended to decrease with increasing feeding frequency at each feed type, and were higher at EP diet than at MP diet. Proximate compositions of fish were not affected by feed type whereas they were significantly affected by various feeding frequencies. As feeding frequency increased, moisture content increased and lipid content increased. It is concluded that three meals a day is effective for improving growth performance of juvenile flounder grown from about 10 g to 60 g.

**EFFECTS OF FEEDING FREQUENCY AND DIETARY COMPOSITION ON GROWTH
AND BODY COMPOSITION OF JUVENILE ROCKFISH *Sebastes schlegeli***

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A 8-week feeding experiment was conducted to determine the optimum feeding frequency with different formulated diets on growth, feed utilization and body composition of juvenile rockfish *Sebastes schlegeli*. Three replicate groups of the fish (average weight of 4.1 g) were fed the experimental diets containing high carbohydrate (HC), high lipid (HL) and high protein (HP) at different feeding frequencies (two meals a day, one meal a day or one meal every 2 days).

Weight gain of the fish fed HC, HL and HP diets were affected by feeding frequency, but not by dietary composition. Weight gain of the fish fed one meal every 2 days was lower than that of other groups. Daily feed intake of fish was significantly affected by both dietary composition and feeding frequency. Daily feed intake of fish fed the HC diet was higher than that of the fish fed the HL and HP diets at the same feeding frequency. Feed efficiency and protein efficiency ratio were affected by both dietary composition and feeding frequency, and decreased with increasing feeding frequency. Whole body lipid content of fish fed the HL diet was higher than that of fish fed the HL and HP diets at the same feeding frequency. Proximate composition of muscle was not influenced by both dietary composition and feeding frequency. These results indicate that one meal a day is more effective than two meals a day or one meal every 2 days, regardless of dietary composition, and high lipid diets have more beneficial effects compared to high carbohydrate diets for improving growth performance and feed utilization of juvenile rockfish weighing between 4 g and 21 g.

**THE USE OF MEAT MEAL AS A DIETARY PROTEIN SOURCE REPLACING FISH MEAL
FOR JUVENILE ROCKFISH *Sebastes schlegeli***

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This study examined the partial replacement of the fish meal with commercial meat meal in practical diets for juvenile rockfish *Sebastes schlegeli*. Five experimental diets were prepared to contain meat meal at 0% (control), 10%, 20%, 30% and 40% with substituting the mackerel meal in the control diet. Three replicate groups of fish (initial mean weight 4.1 g) were hand-fed to visual satiation two times daily for 8 weeks.

Survival was over 90% and was not significantly different among treatments. The best weight gain, feed efficiency and protein efficiency ratio were obtained from fish fed the diets containing 0% and 10% meat meal, and were not significantly different to those of fish fed diet containing 20% meat meal. Condition factor, visceralsomatic index and hepatosomatic index were not influenced by dietary meat meal levels. The contents of moisture, crude protein, and ash of whole body were not significantly affected by dietary meat meal levels, whereas crude lipid content of fish fed the diets containing 30% and 40% was lower than that of fish fed the control diet. Concentrations of plasma total protein and glucose were not significantly affected by dietary meat meal levels. The data obtained in this study indicate that a diet containing up to 20% meat meal could be used for least-cost formulation in juvenile rockfish.

**EFFECTS OF TYPE OF DIETARY CARBOHYDRATE AND α -STARCH LEVEL ON
GROWTH AND BODY COMPOSITION OF JUVENILE FLOUNDER *Paralichthys
olivaceus***

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A 7-week feeding trial was conducted to investigate the effects of type of dietary carbohydrate and α -starch level on growth and body composition of juvenile flounder. Triplicate groups of fish (average weight, 1.5 g) were fed the experimental diets containing 15-25% α -potato starch, 15% β -corn starch and 15% dextrin.

Survival was not affected by type of dietary carbohydrate and α -starch level. The best weight gain was obtained from fish fed the diets containing 15% dextrin. Weight gain of fish fed the diet containing 20% α -potato starch was significantly higher than that of fish fed the diets containing 15% and 25% α -potato starch levels. Feed efficiency and protein efficiency ratio (PER) of fish fed the diets containing 15% β -corn starch were significantly lower than those of other groups. PER tended to increase with increasing α -potato starch. Daily feed intake of fish fed the diet containing 15% β -corn starch was significantly higher than that of other groups. Hepatosomatic index, condition factor and proximate composition of whole body were not affected by dietary carbohydrate. These results indicate that juvenile flounder are able to efficiently utilize dextrin compared to α - and β -starch in diets and that α -potato starch could be incorporated up to 20% in the diet for optimum growth of juvenile flounder.

EFFECTS OF VARIOUS DIETARY LIPIDS ON FISH GROWTH AND QUALITY OF TILAPIA FILLETS

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The present study investigated the impacts of different lipid sources in red hybrid tilapia diet on growth and fillet quality. Tilapia was fed five diets containing 8% added fish oil (FO), fish oil + crude palm oil (1:1), linseed oil (LSO) + crude palm oil (1:1), crude palm oil (CPO) or soybean oil (SBO) for 5 months.—

There were no significant effects ($P>0.05$) of dietary treatment on final fish weight, mortality, growth rate, feed conversion rate, carcass and fillet yield, condition indices and haematocrit or tissue histology. After 1 month into the feeding trial, fatty acid profile in muscle reflected the profile found in diet. No significant differences in proximate composition were observed between groups. The muscle of tilapia fed with the FO diet had significantly higher concentration of n-3 fatty acids such as EPA (20:5n-3) and DHA (22:6n-3). Generally, there was no significant difference between the fatty acid profiles of muscle between 1 month and 5 months on the experiment diets. However the levels of 22:6n-3 in fish liver sample decreased significantly after 5 months compared to that found after 1 month on the diets.

With regards to quality parameters, there were no differences in water holding capacity and texture among fish on various dietary treatments. Sensory analysis values did not differ in response to dietary lipid with the exception of juiciness in frozen tilapia fillet. Fillet from fish fed LSO+CPO were given higher values for juiciness by the sensory panel. During the storage period, the percentage of thaw drip and texture tended to increase although these were not significantly different. Lipid oxidation from all treatments increased after freezing for 6 months as storage time increased. No change in proximate composition and fatty acid profile was detected during the 6 month frozen storage.

In conclusion, this study shows that tilapia diet where fish oil is replaced by CPO can produce similar results with respect to growth, feed efficiency and fish quality. Considering the lower cost and availability of palm oil in Malaysia, it will make it a potential alternative for substituting fish oil in fish feed.

EFFECTS OF KELP ASCOPHYLLUM NODOSUM SUPPLEMENT IN DIET ON GROWTH, SURVIVAL AND RESISTANCE TO DISEASE IN BLACK TIGER SHRIMP *Penaeus monodon*

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Effects of kelp *Ascophyllum nodosum* added diets on *Penaeus monodon* were tested in two experiments. All experimental diets consisted of 40% protein and 10% fat. *A. nodosum* meal was mixed to shrimp basal diet at concentrations of 0, 5 and 10% for the experiment-1 and 0, 2.5, 5, 7.5 and 10% of the diet for the experiment-2. Experiment-1 was tested in *P. monodon* postlarva-15 at an experimental period of 28 days a closed recirculating water system described by Menasveta et.al (1991). Shrimp were evaluated for length, weight and growth rate every week. The result showed that shrimp length fed 5% kelp added diet was higher than those of shrimp fed 10% kelp added diet, but not different from those of the control (0% kelp). Final weight and growth rate of shrimp in each diet were similar (Table 1). In experiment-2, 1.5 month-old shrimp was tested at a period of 56 days in 2x2x1.5 m³ cage culture. Weight and length of shrimp were determined every 2 weeks and at the end of the experiment, survival and resistance of diseases, *Vibrio harveyi* were determined. The results showed that shrimp fed 5% kelp added diet was higher than those shrimp fed 2.5, 7.5 and 10% kelp added diets. There was no significant difference of length and survival rate of shrimp fed different kelp added diets (Table 2). For disease resistant test to *V. harveyi* there were no significant in shrimp's survival rate, total hemocytes count and phenoloxidase activity among the diets.

Table 1. Length, weight and growth rate of postlarval black tiger shrimp.

Diet formula (%kelp)	Day-0		Day-28		Growth rate (%)
	length (cm.)	weight (g)	length (cm.)	weight (g)	
0	1.66±0.18	0.074±0.011	2.73±0.58 ^{ab}	0.121±0.112	63.72
5	1.66±0.17	0.08±0.017	2.83±0.64 ^a	0.141±0.14	76.13
10	1.69±0.14	0.08±0.012	2.64±0.65 ^b	0.129±0.149	61.69

Table 2. Length, weight and survival rate of black tiger shrimp reared in cages with different kelp added diets.

Diet formula (% kelp)	Day-0		Day-56		Survival rate (%)
	length (cm.)	weight (g)	length (cm.)	weight (g)	
0	7.81±0.66	3.79±0.94	9.68±1.15	7.62±2.74 ^{ab}	76.00±8.41
2.5	7.81±0.66	3.79±0.94	9.60±1.11	7.53±2.60 ^b	86.33±4.16
5	7.81±0.66	3.79±0.94	9.77±1.00	8.00±2.32 ^a	79.50±2.89
7.5	7.81±0.66	3.79±0.94	9.52±1.08	7.23±2.40 ^b	77.75±4.79
10	7.81±0.66	3.79±0.94	9.61±1.13	7.40±2.43 ^b	76.25±7.41

Note: means with the same superscript indicate no significant difference ($p>0.05$)

EFFECTS OF ASTAXANTHIN AND VITAMIN A SUPPLEMENTATION ON GROWTH, SURVIVAL AND STRESS TOLERANCE OF *Penaeus monodon* LARVAE

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Penaeus monodon larvae (zoea, mysis and postlarvae) were fed 9 different diets consisting three levels of astaxanthin (AX; 0, 200 and 500 ppm) and three levels of vitamin A (A; 0, 20,000 and 40,000 IU/kg). The experiment was 3x3 factorials in a completely randomized design with three replicates. Diets were practical forms with mean protein and lipid of 50 and 11%, respectively. The results showed that Ax and A had interactive effect on zoeal and mysis survival and growth rate. Shrimp's larval fed AX 500 ppm provided the highest survival and growth rate. For the postlarval stage, larvae fed only AX 500 ppm had higher survival rates than those shrimp fed other diets. The growth of postlarvae fed diet without addition of Ax and A was significantly greater than those of shrimp fed other diets. Shrimp fed A 40,000 IU/kg showed less survival rate in zoeal stage, but in mysis and postlarval stages, shrimp fed higher AX and A contents gave less survival rate than those shrimp fed only A diets. During a low salinity stress test, PL15 shrimp fed diets containing AX 500 ppm had significantly greater tolerance to low salinity exposure.

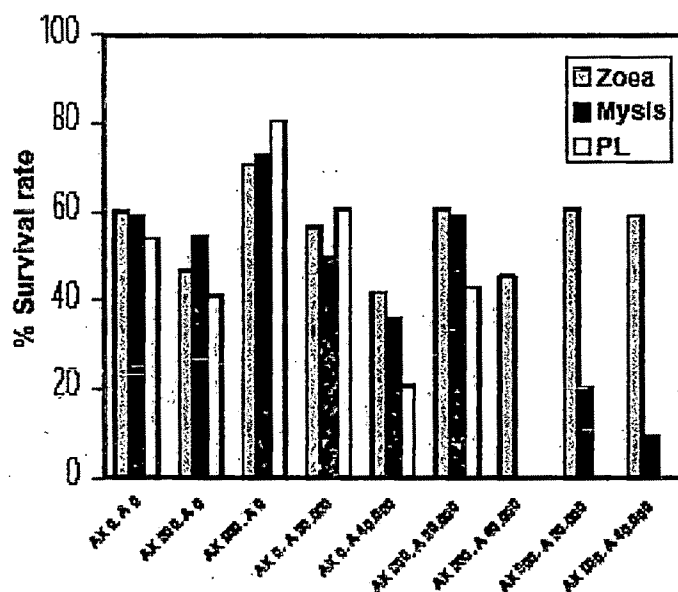


Figure.1 Survival rate of different larval stage of *P. monodon* fed diet supplemented with different concentrations of astaxanthin (Ax) and vitamin A (A).

EFFECTS OF VITAMIN E AND ASTAXANTHIN ON MATURATION OF MALE WHITE SHRIMP *Litopenaeus vannamei*

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This study was to determine effects of dietary vitamin E and astaxanthin on reproductive performance of male white shrimp *Litopenaeus vannamei*. The experiment was conducted using 3x3 factorials involved randomized completely design. Three level of vitamin E (200, 400 and 600 ppm) and 3 levels of astaxanthin (100, 300 and 500 ppm) combinations were prepared from the same basal diet. Five month-old *L. vannamei* males were selected from intensive rearing pond. At the beginning, only shrimp with no appearance of spermatophores were used for the experiment. The feeding experiment was operated 40 days in 4 replications. The results showed no interactions between dietary vitamin E and astaxanthin on growth, survival and reproductive performances (spermatophore weight, no. of sperm/spermatophore, percentage of live and abnormal sperm). Both vitamin E and astaxanthin had no significant effect on growth ($P>0.05$). Astaxanthin had also no significant effect on survival rate of the shrimp. For vitamin E, shrimp fed diets supplemented with 600 ppm vitamin E had significantly higher survival rate than those fed 200 ppm ($P<0.05$). Effects on reproductive performances: significantly higher mean of spermatophore weight was observed in shrimp fed diets with vitamin E 400 ppm, compared to those fed diets with vitamin E 200 ppm ($P<0.05$), while astaxanthin at 500 ppm provided significantly higher spermatophore weight than those shrimp fed diet containing 300 ppm astaxanthin ($P<0.05$). For sperm counts, all levels of astaxanthin showed no significant effect on number of sperms per spermatophore ($P>0.05$) but vitamin E at 400 ppm had significantly higher sperm number than those fed with vitamin E 200 and 600 ppm ($P<0.05$). Shrimp fed with vitamin E 400 and 600 ppm had significantly greater live sperms than those fed 200 ppm ($P<0.05$) while astaxanthin showed no effect on live sperms ($P>0.05$). Percentage of abnormal sperms investigated as percentage of abnormal body sperm indicated no significant effect of both vitamin E and astaxanthin, when evaluated in term of percentage of abnormal spike, vitamin E enriched diets did not show any significant difference but shrimp fed diets containing astaxanthin 300 and 500 ppm had significantly lower abnormal spike than those fed diet containing 100 ppm of astaxanthin ($P<0.05$). The present study suggested that supplementation of vitamin E and astaxanthin in shrimp diets as appropriate amount can enhance reproductive performance in male white shrimp *L. vannamei*.

Table showing the results of astaxanthin and vitamin E on survival and reproductive performances of male white shrimp *Litopenaeus vannamei*

Parameters	Dietary astaxanthin concentrations (ppm)			Dietary vitamin E concentrations (ppm)		
	100	300	500	200	400	600
Survival rate (%)	97.917±7.0502 ^a	98.875±5.446 ^a	100±0 ^a	95.833±9.617 ^a	98.956±5.103 ^{ab}	100±0 ^a
Spermatophore weight (g)	0.0406±0.0164 ^{ab}	0.0393±0.0117 ^b	0.0467±0.0126 ^a	0.0344±0.0091 ^b	0.0439±0.0162 ^a	0.0416±0.0116 ^{ab}
Sperm counts (x 10 ³ cells/spermatophore)	0.965±0.471 ^a	1.031±0.473 ^a	1.056±0.500 ^a	0.774±0.406 ^b	1.267±0.519 ^a	1.001±0.380 ^b
Live sperms (%)	93.353±4.533 ^a	92.971±6.242 ^a	93.476±8.016 ^a	90.909±7.442 ^b	93.417±6.054 ^a	95.479±2.667 ^a
Abnormal spike of sperm (%)	35.015±11.653 ^a	29.302±9.683 ^b	26.137±9.730 ^b	30.819±10.450 ^a	31.107±12.600 ^a	31.128±11.430 ^a

GROWTH ENHANCEMENT EFFECT OF PLANT EXTRACTS ON CULTURED FISH

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Various kinds of oriental herbs are known to promote growth rate of animals, including humans. The study investigated the growth enhancement effect of selected plant extracts using several cultured fish species. Through a series of preliminary tests, *Eleutherococcus senticosus* (Siberian Ginseng), *Artemisia capillaris* (Chinese wormwood) were found to be effective. Then, field trials were conducted in aquaculture farms, with a mixture of these 2 plant extracts. As a result, growth enhancement effects were observed in carp (*Cyprinus carpio*), rockfish (*Sebastes schlegeli*), olive flounder (*Paralichthys olivaceus*) and mullet (*Mugil cephalus*).

Growth hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) of rat were measured, after 3 months' administration of the plant extracts. In female experiment groups, levels of GH and IGF-1 were significantly higher than those of control groups. The levels of GH and IGF-1 in male experimental groups were also higher than those of control groups, although there was no significant difference between two groups. It is suggested that these oriental herbs may stimulate pituitary glands to secrete GH and IGF-1, and consequently enhance growth.

The present study indicated that growth enhancement can be achieved by administration of oriental herbs orally. More detailed studies should be undertaken to clarify the mechanism(s) lying in the enhancement of fish growth.

* : Significantly different from control group ($P < 0.05$)

Fish species	Parameter	Before experiment	Control group after experiment	Experimental group after experiment
<i>M. cephalus</i>	Ave. Length (mm)	90.9 ± 7.5	129.8 ± 6.9	142.5 ± 10.2
	Ave. Weight (g)	11.3 ± 2.9	33.6 ± 5.7	44.5 ± 10.4
<i>P. olivaceus</i>	Ave. Length (mm)	10.2 ± 1.3	12.3 ± 1.9	13.7 ± 1.9*
	Ave. Weight (g)	13.4 ± 2.3	16.7 ± 2.4	22.7 ± 2.1*
<i>S. schlegeli</i>	Ave. Length (mm)	12.3 ± 1.3	13.4 ± 1.2	14.7 ± 1.7
	Ave. Weight (g)	27.5 ± 3.7	34.2 ± 2.9	45.7 ± 3.1*

UTILISATION OF SPENT BLEACHING CLAY FROM PALM OIL REFINING AS A FEED INGREDIENT IN TILAPIA DIETS

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Spent bleaching clay (SBC) from the palm oil refining industry was included in pelleted feed for the tilapia, *Oreochromis* sp., to determine the influence of SBC on growth performance and feed utilization efficiency. Hematocrit and hepatosomatic index (HSI) values were also determined for fish fed the different diets. An 8-week feeding trial was carried out. Five isonitrogenous (30% crude protein) and isocaloric (12.55kJg^{-1}) diets with 13% lipid and containing 0%, 10%, 20%, 30% and 40% SBC respectively were formulated. Each experimental diet was fed to triplicate groups of 12 tilapia with an initial mean weight of $3.90 \pm 0.01\text{g}$. No significant differences ($P>0.05$) were observed on the growth performance of tilapia. However, significant differences ($P<0.05$) were observed in the feed utilization efficiency of fish fed the various diets. Mortality rate was low and this showed that SBC is safe to use. Hematocrit and HSI values for fish fed the different diets were not significantly different ($P>0.05$). The inclusion of 40% SBC diet gave the highest pellet stability. In conclusion, this study showed that SBC which contains a substantial amount of absorbed oil through the refining process of palm oil is a potential source of dietary lipid and energy in pelleted diets for tilapia. The use of SBC in fish diets could help reduce environmental problems caused by the disposal of the waste clay as well as reduce the reliance on fish oil and reducing cost of commercial fish feeds in the process.

ANTIOXIDATIVE ACTIVITY FROM MICROALGAE OF MALAYSIAN WATERS

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Search for natural antioxidants are mainly focused on higher plants. Little has been done on microalgae even though microalgae contribute 25% of the total vegetation on the planet. Lipid peroxidation has been suggested to occur through involvement of free radical that attacks polyunsaturated fatty acids in membranes and plasma lipoprotein that caused oxidative damage to biological cells. The antioxidative activity of microalgae that can inhibit lipid peroxidation is probably due to the active compounds present in the microalgae. In this study, the antioxidative activity of extracts from various purified microalgae found in Malaysia was evaluated. Thirteen microalgal extracts were screened for antioxidative activity using Ferric Thiocyanate (FTC), Thiobarbituric Acid (TBA) and 2, 2'-di-phenyl-1-picrylhydrazyl (DPPH) methods. The inhibitory activity of the sample against lipid peroxidation and the antioxidant potential of each samples were evaluated.

Out of 13 samples screened, only five extracts were active for antioxidative activity meanwhile others were inactive including two freshwater microalgae. Results showed that every active sample tested were active but tend to exhibit high or low antioxidative activity depending on solvent used to extract it. The prymnesiophyte *Isochrysis galbana* showed high antioxidative activity in both methanol and water extracts. Meanwhile, green algae *Nannochloropsis oculata* and *Tetraselmis tetrahele* showed to have more active antioxidative activity in water extracts than methanol extracts and diatom *Chaetoceros calcitrans* showed to have high active antioxidative activity when extracted with methanol than water extracts (Table). Thiobarbituric Acid (TBA) method determines lower molecular compound's peroxides and measures its relative concentration. This proven the antioxidative activity in the samples tested meanwhile 2,2'-di-phenyl-1-picrylhydrazyl (DPPH) methods showed the Antioxidant Potential of the microalgae used.

Table Antioxidative activity of different marine microalgae in different types of extraction. Lower absorbance (500 nm) than standard (Quercetin) resulted in higher antioxidative activity using FTC method and TBA.

Activity (≤ Quercetin)	Antioxidative level		
	Most active (<Quercetin)	Average (=Quercetin)	Low (>Quercetin)
Methanol	<i>Isochrysis galbana</i> <i>Chaetoceros calcitrans</i>	<i>Nannochloropsis oculata</i>	<i>Tetraselmis tetrahele</i>
Water	<i>Tetraselmis tetrahele</i> <i>Nannochloropsis oculata</i> <i>Isochrysis galbana</i> <i>Chlorella sp.</i>	Nil	<i>Chaetoceros calcitrans</i>

RELATIONSHIP BETWEEN EGG QUALITY AND MATURITY OF OVULATED EGG IN JAPANESE EEL *Anguilla japonica*

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The Japanese eel, *Anguilla japonica*, is an important species in the aquaculture industry of Japan, China and Korea. Recently, success has been achieved in the production of glass eel in captivity. However, the fertilization and hatching rates are still highly variable. Thus, improvement of egg quality is necessary for consistent mass production. In this study, the oocyte maturation related proteins and the stage of meiotic division in ovulated eggs from artificially matured eels were examined, to assess whether these parameters are related to the egg quality. *A. japonica* females received weekly injections of salmon pituitary homogenate. To induce ovulation, matured fishes were given an injection of 17 α -hydroxyprogesterone. The fertilization and hatching rates were estimated in each ovulated fish. Ovulated eggs were analyzed by SDS-PAGE and by Western blotting using anti-goldfish cdc2 and anti-Bufo cyclin B antibodies. In addition, ovulated eggs were fixed for histological observation of stage of meiotic division. The anti-cdc2 and anti-cyclin B antibodies recognized three (34, 35, 37 kDa) and two (52 and 50 kDa) bands, respectively, in ovulated eggs. Especially, cyclin B and 35kDa cdc2 were found in high fertilizable eggs, but not in low fertilizable eggs. Various stages of meiotic division were observed in ovulated eggs of Japanese eel. Low fertilizable eggs were mainly composed of germinal vesicle migration (GVM) egg and Prometaphase I. On the other hand, high fertilizable eggs were mainly composed of Metaphase II. The presence of abnormal meiotic division and overripe eggs seemed to affect the viability of other eggs. From the result of this study, stages of meiotic division and oocyte maturation related proteins may serve as indicators of egg quality for artificially matured Japanese eel.

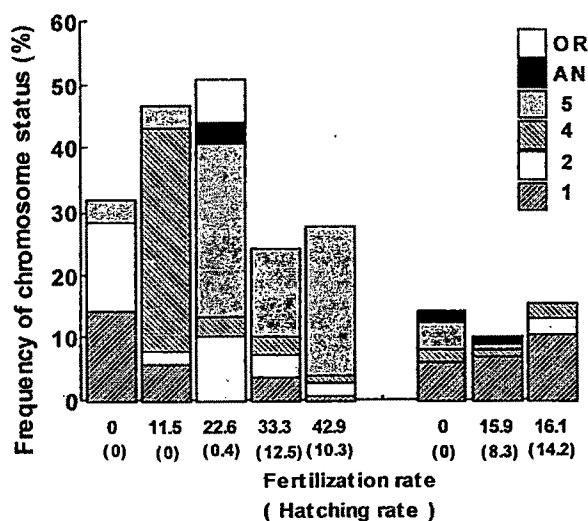


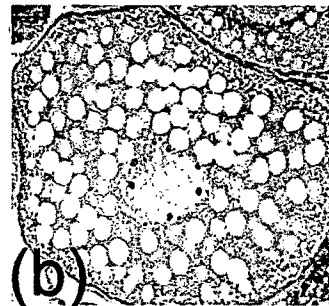
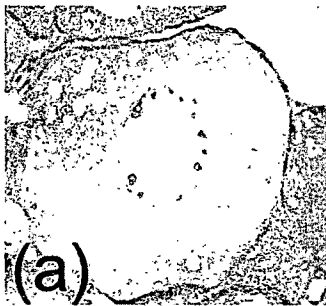
Figure 1 Relationship between frequency of chromosome status and fertilization rate or hatching rate. 1, GVM egg; 2, Prometaphase I; 3, Metaphase I; 4, Anaphase I ~ Prometaphase II; 5, Metaphase II; AN, Abnormal meiotic division; OR, Overripe egg.

INDUCTION OF EARLY OOCYTE GROWTH BY THE ORAL ADMINISTRATION OF ANDROGEN IN JAPANESE EEL *Anguilla japonica*

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In a previous study, the authors implanted 11-ketotestosterone (11KT), a major androgen in some fish, and estradiol-17 β (E2) by the silastic capsules in immature female Japanese eels, *Anguilla japonica*, that have ovaries containing previtellogenic oocytes of about 150 μ m in diameter. After one month, the oocyte diameters of eels treated with 11KT but not E2 increased significantly with an increased number of oil droplets. In the present study, the effects of oral administration of other androgens, 17 α -methyltestosterone (MT) and testosterone (T), and E2 on the previtellogenic oocyte growth was examined. Firstly, female eels that have oocytes of about 40 μ m in diameter were fed the diets containing MT or E2 at the dose of 0, 0.1 or 1.0 mg/kg-diet for two and four months. However, significant differences were not observed between the oocytes in control and those in hormone treated groups. Subsequently, female eels that have oocytes of about 90 μ m in diameter were fed the diets containing MT, T or E2 at the dose of 0 or 1.0 mg/kg-diet during the same period. After four months, oocyte diameters in the MT treated group increased with greater abundance of oil droplets than those in other groups. These results indicate that the oral administration of MT can induce growth of previtellogenic oocytes over 90 μ m in diameter in Japanese eel.



**COMPARATIVE GONAD DEVELOPMENT, GROWTH AND BODY COMPOSITION OF
GRASS CARP AND FEMALE GRASS CARP × MALE BIGHEAD CARP HYBRID**

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Grass carp and female grass carp × male bighead carp hybrids were reared under polyculture conditions in combination with silver carp, bighead carp and common carp during 4 months (Jul-Nov). Physicochemical parameters of pond water as well as growth, gonad development and carcass proximate analysis were compared between grass carp and hybrid fishes.

Final results showed that the condition factor of hybrid and grass carp decreased with means of 1.07 and 1.32, respectively. Final weight gain of 37.8 in hybrid and 37.7 in grass carp (no significant differences), specific growth rate in both species were 0.25, while the daily growth rate was found to be 3.44 g. and 0.44 g. in grass carp and hybrid, respectively.

Histological studies of gonad development in both species showed that, the ovaries of grass carp had a normal growth and consist of primary oocyte, but hybrid ovary growth remained in the oogonia primary stage. In male grass carp, testis has a proliferated spermatogonia stage in contrast with primary spermatocyte stage in hybrid testis. Proximate analysis of carcass of both species showed no significant differences on protein, ash, lipid and moisture content of meat ($p>0.05$).

INDUCED SPERMATION OF RAINBOW TROUT *Oncorhynchus mykiss* USING A GNRH ANALOGUE

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In this study the benefits of using the first Iranian made GnRHa (D-Ala6 desGly10) mGnRH ethylamid to induce spermiation in male rainbow trout, *Oncorhynchus mykiss*, was evaluated. In addition, its effect on acceleration and synchronization, quantity and quality of milt and plasma testosterone (T) levels were also examined.

For these purposes, 40 non-spermiating male *O. mykiss* were injected with a mammalian gonadotropin releasing hormone analog GnRHa preparation of 0 (control), 30, 40, 80 µg/kg body weight (B.W) or vehicle (propylen glycol). Spermiation was synchronous and accelerated in treated groups. Six days after first injection, the cumulative spermiation rates reached respectively 40, 49 and 79% in injected groups (i.e. 30 to 80 µg/kg B.W.) while none of the control fish spermiated. GnRHa injections advanced spermiation and reduced the average time to spermiation from 16 ± 3.67 days for control group to 14.3 ± 2.2 , 9.2 ± 0.75 and 6.6 ± 0.29 days for treated groups, respectively ($p < 0.05$). The average volume of total expressible milt increased significantly after treatment with GnRHa from 8.29 ± 1.59 ml/kg B.W. for control to 11.42 ± 1.55 , 14.39 ± 1.55 and 17.14 ± 1.55 ml/kg B.W. in groups 2 to 4 respectively ($p < 0.05$). The fertilization and survival rates to the eyed stage did not show any significant differences among the groups ($p > 0.05$). Circulating levels of testosterone (T) prior to the GnRHa treatment were relatively low in all groups. Treatment with GnRHa induced significant increase in plasma T after 12 h, increasing it to 43.4 ± 5.82 , 38.66 ± 5.63 , 39.72 ± 5.07 ng/ml in groups 2 to 4 respectively, which were higher than T levels for control (i.e. 24.58 ± 7.13 ng/ml, $p < 0.05$). These levels remained high until 48 h in treated groups; but after this time, T levels reduced to the basal levels of time 0, except in group 4 which had received its second GnRHa injection at time 48.

RELATIONSHIP BETWEEN OVARIAN DEVELOPMENT AND SERUM LEVELS OF GONADAL HORMONES IN THE PROTOGYNOUS FISH *Epinephelus coioides* IN THE PERSIAN GULF WATER

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Epinephelus coioides (Family Serranidae) is protogynous. This species is one of the most important fishes in food chain of marine proteins of Persian Gulf.

In this study, monthly samples of *Epinephelus coioides* were examined during 17 months from May 2001 to September 2002 in the Persian Gulf. Blood samples and oocytes at all stages of development were taken from *Epinephelus coioides*. The hormones have assayed and also different stages of gonads from the histological point of view were studied by light microscope.

The results indicated that change in serum levels of gonadal estradiol were correlated with ovarian development. Serum 17-*B* estradiol level increased in April reached their highest levels (1.482 ng/ml $P < 0.01$) in ripe females whose gonads contained both cortical alveoli and vitellogenic oocytes during the spawning season. 17-*B* estradiol level was also correlated with GSI. The gonadosomatic index (GSI) were highest in March–May. Peak GSI occurred in May. Testosterone peaked in female during vitellogenesis and maturation (1.045 ng/ml $P < 0.01$). Plasma concentration of 17,20BP was significantly elevated in female with ovaries undergoing final oocyte maturation (FOM) and to have high potency to induce maturation.

Regarding to the results of this research, it seems that the period of spawning in *Epinephelus coioides* is in May - June and the aquaculture procedure of *Epinephelus coioides* could be performed in the above mentioned periods.

**DIFFERENCE IN CARNOSINASE ACTIVITY AS RELATED TO CARNOSINE
DEGRADATION BETWEEN JAPANESE AND EUROPEAN EELS**

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A high-performance liquid chromatographic (HPLC) procedure was used to determine the contents of L-histidine (His), carnosine (β -alanyl-L-histidine, Car), and anserine (β -alanyl-L-1-methyl-L-histidine, Ans) in the tissues and plasma of cultured Japanese eel (*Anguilla japonica*) and European eel (*Anguilla anguilla*). His and Car were found in the muscle, vertebrae and gill of both eels, but not detected in the liver and plasma. Ans was not detected in any tissues and plasma. The muscle contained the highest amount of Car among the tissues. Japanese eel had a higher Car content than European eel.

Car in European eel meat was rapidly degraded at the initial stage during storage at 5 and 25°C. The constituent amino acids of Car, His and β -alanine (β -Ala) were increased. The increased moles of His and β -Ala were almost equal to the decrease of Car. This result showed that Car was rapidly converted to β -Ala and His in European eel meat after storage. However, no significant change in Car level ($p>0.05$) of Japanese eel meat was found during storage. The Car was still abundant even when the fish was spoiled, and β -Ala and His were slightly increased. His in European eel meat increased at the initial stage and then decreased during storage at 25°C. As His decreased dramatically during elongated storage, histamine (Him) rapidly increased. The increased mole was almost equal to that of the decrease in His. The result showed that Car in *A. anguilla* meat was converted to His and then further to Him. However, His remained unchanged during storage at 5°C.

The carnosinase activity measured by fluorescence method showed that the activity was found in different tissues and plasma of both eels except for the muscle. The activity in order was liver > gill > plasma > vertebrae > muscle. The optimal pH and temperature of tissue carnosinase were 9.5 and 40°C, respectively. Plasma carnosinase was stable at the pH values ranging from 3.5 to 8.5, and the temperature from 0 to 60°C. The activities of plasma and tissues in *A. anguilla* were higher than those in *A. japonica*. Carnosinase was present in the meat of the former, but not detected in the latter. This result revealed that the reason why Car in *A. anguilla* meat was more rapidly degraded than that in *A. japonica*. After bleeding treatment, the degradation of Car in *A. anguilla* meat still occurred during storage at 5 and 25°C; however, the rate was lower than that without bleeding.

**A PRELIMINARY STUDY OF PHYLOGENETIC RELATIONSHIPS OF THE 'Labeoin'
GROUP OF FISHES REVIEWED BY MITOCHONDRIAL 16S rRNA SEQUENCES**

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The Labeoin is a group of fishes commonly found in Malaysia and throughout Southeast Asian region. However, controversy still remains regarding the taxonomy of this group. Phylogenetic relationships among four species within this group namely *Labio barbus festivus*, *L. leptocheilus*, *Osteochilus hasselti* and *O. vittatus* were well resolved based on DNA sequencing of the 16S rRNA mitochondrial gene. Neighbor joining and parsimony analyses conducted using PAUP* Version 4.0b10 showed that most of the specimens were classified accordingly to their species except one specimen of *O. vittatus* which was isolated from its own cluster suggesting that this sample was misidentified at the early stage and it might be a new species. Taking these findings into consideration, misidentification and wrong classification could be detected. Finally, it is hoped that this findings will contribute towards the correct taxonomic status of this group.

THE ANALYSIS OF CANVAS TANK REAR TILAPIA (CTRT) AND POND REAR TILAPIA (PRT) & THE PRODUCTION OF CTRT 'SOLOK LADA'

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A preliminary study on the sensory evaluation of Canvas Rear Tank Tilapia (CTRT) samples treated with different feeding rates (satiation fed with feeding rate 3X a day, 6% of body weight with feeding rate twice a day, 6% of body weight with feeding rate 3X a day, 4% of body weight with feeding rate twice a day, 4% of body weight with feeding rate 3X a day) was carried out. Different feeding rates were found not to have influence on the taste and odour (fishy) of the samples. The satiation fed (produce bigger fillet size) was then choose to compare with the Pond Rear Tilapia (PRT) samples.

Fish samples (CTRT and PRT) were processed in the form of fish fillets then subjected to different types of washing (I) washing with normal plain water (II) washing with tamarind water, prior to difference cooking procedures; frying and steaming. Proximate analysis, sensory evaluation and texture analysis were carried out in order to see the differences between two samples of fish from different habitat. The edible portion of CTRT contains 79% moisture, 13.4% protein, 4.73% fat, 0.9% ash and 0% crude fibre. While PRT consist of 76.5% moisture, 11.2% protein, 3.3% fat, 2.88% ash and 0.1% crude fibre. The results from analysis of varians showed that there was no significant different ($p>0.05$) in term of texture and odour of both fish fillet washed with tamarind water. However, there was a significant different ($p<0.05$) for texture and odour of both fillets washed with normal plain water. Nevertheless, result from the Texture Analyzer showed that there were differences in texture for both fish fillet for all the treatment.

The same CTRT samples were also used to produce a local Malaysian dish called 'Solok lada' as a comparison to marine fish 'kembung'. The samples were subjected to the washing treatment with tamarind water prior to steaming procedure. Comparisons were made based on proximate anaiysis and sensory evaluation by trained panelists. The results showed that CTRT 'solok lada' was more accepted due to its sweeter taste and whiter flash colour compared to the 'kembung solok lada'. However, proximate analysis showed no significance difference in nutritional values for both products.

The effect of two storage temperatures (10°C and -18°C) on the CTRT 'solok lada' were also studied. The result showed that 'solok lada' stored at -18°C had a longer shelflife (75 days) compared to 'solok lada' which was stored at 10°C (28 days). However, chilling injury was observed for the green chili of the 'solok lada' stored at -18°C. The result from the proximate analysis showed an increase in the fat content and decrease in the protein content, after 75 days of storage at -18°C. Nevertheless there was no significance difference found in the crude fibre, moisture, total ash and carbohydrate content.

THE EFFECT OF SEAFRESH SOLUTION ON QUALITY CHARACTERISTICS OF WALKING CATFISH *Clarias batrachus*

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Today, the fish industry has become one of the largest industries in the world. There is a great market potential for and seafood products. One of the factors limiting the successful development of this industry in Malaysia is the quality of landed fish. Moreover, it has to meet the quality requirements of not only retailers but consumers as well. *Seafresh* solution is a natural 100% organic product, which is biotechnologically derived from local plant sources. Use of this solution was introduced as an alternative preservative for fish and seafood products. Thus, studies on quality characteristics of walking catfish (*Clarias batrachus*) were carried out using *Seafresh* solution at different concentrations (0 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm). The fish samples were soaked in *Seafresh* solution for 10 minutes. Then, the samples were stored in a styrofoam box containing ice in a ratio of 1:2 (fish:ice) and stored in cold room (10° C) for 14 days. On every alternate day, the ice water was drained and replenished with new ice whilst sampling of fish was carried out at the same time.

Microbiological analysis was performed for total mesophilic and psychophilic aerobic counts as well as coliform test. These analyses were done using Plate Count Agar (OXOID) and McConkey broth (DIFCO). Biochemical analysis was also carried out for pH profile using pH meter (Jenway model 3305) and sensory evaluation was also done to observe the organoleptic changes throughout storage. Results from the experiment showed that there was an increment in the total mesophilic aerobic counts, from log 4.04 CFU/g to log 7.15 CFU/g. However, there was no growth of psychophilic bacteria after 7 days of storage at 10°C. For coliform test there was an increment, from log 4.86 CFU/g to log 6.38 CFU/g in the control sample without the solution but negative results were obtained for all treated samples. Biochemical results for pH showed similar trends of increasing values and were closely related to the microbiological data until spoilage set in. For overall changes in sensory scores of colour, odour, texture and overall acceptability, there is no significant differences ($p>0.05$) if compared between control with 25 ppm and control with 50 ppm of *Seafresh* solution. Significant differences ($p<0.05$) were obtained however control with 75 ppm and control with 100 ppm *Seafresh* solution. The present study showed that *Seafresh* solution was effective as an alternate preservative for walking catfish at above 75 ppm levels in removing the muddy earthy flavour as well as bacteriologically against the coliforms. Future study will be carried out on the toxicity of *Seafresh* solution on human use and further role of this solution on other quality characteristics of other fish and seafood products.

PROXIMATE COMPOSITION AND COST PER PROTEIN UNIT OF TRADITIONALLY PRODUCED FISH, SQUID AND SUN-DRIED SHRIMP MEALS OF *Decapterus* sp., *Sepia* sp., AND *Acetes* sp.

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Proximate composition and cost per protein unit of trash fish (*Decapterus* sp.), squid (*Sepia* sp.) and sun-dried sergestid shrimp (*Acetes* sp.) meals produced using traditional method were evaluated. Fish and squid were subjected to four different processes; boiling, squeezing, drying and grinding. Sun-dried shrimp was only subjected to drying and grinding processes. Moisture content was highest in fish meal (13.53 ± 1.58), followed by squid meal (5.79 ± 0.19), and sun-dried sergestid shrimp (4.29 ± 0.47). Sun-dried sergestid shrimp contained high level of ash (17.10 ± 0.93), which is twice greater than ash content of fish and squid meals. There was no significance different ($p > 0.05$) in term of crude lipid content of the meals. Crude protein content of fish and squid meals (43 ± 2.37 and 40 ± 2.42 % respectively) were significantly higher ($p < 0.05$) than sun-dried sergestid shrimp meal (19 ± 0.72 %). Price per protein unit (RM/protein unit) of squid meal (RM1.36) was about three times higher than of fish and shrimp meals (RM0.36 and RM0.40 respectively). Event though dried shrimp meal has lower crude protein percentage, the cost per protein unit is comparable to fish meal and higher than squid meal. Low crude protein content of sun-dried shrimp meal in the study was probably due to the effect of sun-drying process and long storage. Overall, the nutritional content of meals produced, especially crude protein, is heavily affected by processing method, species used, and the quality of raw materials.

OCCURRENCE OF *Vibrio vulnificus* IN FISH AND SHELLFISH FROM MARKETS IN CHINA

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Vibrio vulnificus is a marine and estuarine bacterium naturally occurring in tropical and temperate environments and causes primary septicemia and wound infections in humans. Primary septicemia usually occurs through the consumption of raw or lightly cooked seafood, while wound infections occur by exposure to seawater or the handling of fish.

The occurrence of *V. vulnificus* was investigated in 48 samples consisting of 11 species of live seafood available from markets in coastal cities of China. The homogenized samples were enriched in alkaline peptone water and the cultures were streaked onto mCPC agar plates for the isolation. Yellow and flat colonies were subjected to PCR analysis targeting hemolysin gene of *V. vulnificus*. Direct spreading method was also used for the isolation. The bacteria were detected in 4 out of 4 razor clam, *Sinonovacula constricta*, samples, in 7 of 7 giant tiger prawn, *Penaeus monodon*, samples, and 5 of 9 mantis shrimp, *Squilla oratoria*, samples (Table 1). The maximum level of *V. vulnificus* was found to be 3.4 (log₁₀) CFU/g in the razor clam and 4.9 (log₁₀) CFU/g in the prawn samples by the direct spreading method. The determination of bacterial counts on the prawn body showed that most of the bacteria were found on the shells and very scarcely in the edible muscle. However, it also suggested that dense populations potentially occur in the intestines. These results suggest that *V. vulnificus* could be a potential health hazard to the public consuming the shellfish, and the aquaculture and distribution systems should be improved for food safety.

Table 1 Occurrence of *Vibrio vulnificus* in seafood from markets in China

Sample	City ^a	No. of samples ^b	APW ^c	Direct mCPC ^d	
			Positive	Positive	Log10 (range) ^e
Bivalves					
Razor clam	S	1	1	0	-
Razor clam	S	3	3	3	3.4 (3.2- 3.4)
Little necked clam	S	2	0	0	-
Little necked clam	D	2	0	0	-
Little necked clam	Q	2	0	0	-
Ark shell clam	D	2	0	0	-
Ark shell clam	Q	2	0	0	-
Oyster	D	2	0	0	-
Crustacean					
Giant tiger prawn	S	3	3	3	4.0 (3.9- 4.0)
Giant tiger prawn	S	4	4	4	4.6 (3.1-4.9)
Mantis shrimp	S	4	3	0	-
Mantis shrimp	G	5	2	0	-
Chinese mitten crab	S	2	0	0	-
Chinese mitten crab	S	3	0	0	-

a: S, Shanghai; D, Dalian; Q, Qingdao; G, Guangzhou. b: All samples consisted of three or more individuals. c: Enrichment in Alkaline peptone water and isolation on mCPC. d: Isolation and counting by direct-spreading method using mCPC. e: The values show the averages of *V. vulnificus* viable counts (log₁₀ CFUs/g) and the ranges of those.

**ROLE OF ORGANIZATIONAL CULTURE IN INFLUENCING SUCCESS OF QUALITY
MANAGEMENT SYSTEM IN SEAFOOD INDUSTRY IN KERALA, INDIA**

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An organization's culture is the everyday manifestation of its underlying values and traditions. This in turn reflects the quality culture of the organization. Only firms with a healthy quality culture can become world-class competitors, which is the underlying aim of almost all organizations. This paper therefore aims at studying the role of various organizational culture factors in affecting the success of quality management system in the seafood industry in Cochin, India. The elements of organizational culture, which are analyzed in the study, include the business environment, organizational values, cultural role models, organizational rites, rituals and customs, and cultural transmitters. Leadership styles and management patterns are analyzed. Intrinsic to quality culture is the organizational climate, which is also assessed in the study.

Sampling survey technique with survey instruments of questionnaires and interviews was used to obtain data. Random sampling technique was used to select the plants under study. In-depth study of 5 plants was undertaken, while almost 80% of the EU plants in Cochin were sampled generally.

The findings showed that there is a change in trend from autocratic to participative styles of leadership, with increasing awareness of the role of teamwork. Analysis also revealed that there is an inclination towards continuous improvement and about 6.5% of the plants had effective Quality Circles. About 13% of the plants had quality – oriented incentive systems. Though the organizational culture presently has a higher production – oriented emphasis, the scope for developing a quality – oriented emphasis seems enormous, due to increasing levels of management commitment, as observed.

Future studies will prescribe the type of organization culture appropriate for implementing a total quality management system, which is the ultimate tool for enhancing customer satisfaction.

THE STUDY OF SOME BACTERIAL PATHOGENS IN CULTIVATED *Oncorhynchus mykiss* , *Cyprinus carpio* AND *Hypophthalmichthys molitrix* IN IRAN

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There is an increasing demand for fish products in the world in recent years. Therefore, the hygienic quality of these products is the main concern. With respect to this fact that the water in fish farms may be contaminated by sewage, fish-feed and manure, which is used in fish farms in some of developing countries, and may have a flora of microorganisms of sewage, manure, etc. As a result, there is always the risk of contamination of fish that are produced in these farms.

In this study 40 samples (20 *Oncorhynchus mykiss*, 16 *Cyprinus carpio* and 4 *Hypophthalmichthys molitrix*) were analysed using American Public Health Association (APHA) methods for detection of *Listeria monocytogenes*, *Escherichia coli*, *Vibrio parahaemolyticus* and *salmonella* spp..

Listeria monocytogenes (1/2a) was just isolated from 4 *Cyprinus carpio*. All the samples were coliform positive. From 20 *Oncorhynchus mykiss* 7 samples were *E.coli* positive, while 2 samples out of 16 *Cyprinus carpio* were contaminated with *E.coli*. *Vibrio parahaemolyticus* was isolated from all the samples. Finally 3 *Oncorhynchus mykiss* out of 40 samples were *Salmonella* positive. DNA typing of *Salmonella* positive samples indicated that three of them were *S. tythimurium*. According to the results and with respect to this fact that consumers of these products in northern provinces of Iran prefer to consume them undercook, they may be considered as the risks of food infection for consumers.

GROWTH OF RED TILAPIA HYBRID AFTER PRE-TREATMENT IN CLOVE OIL

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Clove oil is used as an anaesthetic in the handling and transportation of live fish. There were claims that clove oil stunted the growth of Red Tilapia Hybrid. In-tanks culture trials were conducted on two batches of fingerlings. The initial mean weights in Trial I and Trial II were 26.92 ± 5.80 g and 35.66 ± 10.55 g, respectively. At the end of 12 weeks, there were no significant differences ($p < 0.05$) in the final mean weights between the controls and the treatments (30-minutes baths at 60, 80, 100 and 120 ppm clove oil) in both the trials.

COMPARISON IN BIOMASS PRODUCTION AND ECONOMICS BETWEEN PANGASID CATFISH MONOCULTURE AND COMPOSITE CULTURE WITH CARP IN FARMERS' PONDS

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Culture of pangasid catfish, *Pangasius hypophthalmus*, has flourished throughout the country within few years since after its introduction in Bangladesh from Thailand in 1990, and contributed significantly to the country's annual fish production and livelihood of the people. Popularity of farming this fish is in decreasing trend due to increasing problem with high cost of pelleted feed and lower consumer demand and market price due to off-flavour of the fish. To overcome this problem, an experiment with a set of three treatments (T₁, 30000 pangasid catfish/ha; T₂, 15000 pangasid catfish + 15000 silver carp/ha; and T₃, 20000 pangasid catfish + 10,000 silver carp/ha) each with three replications was conducted in farmers' ponds for 14 weeks from 01 May to 6 August. Pelleted feed was given only for the pangasid catfish. A 50% replacement of pangasid catfish by silver carp (T₂) showed higher growth rate, better water quality (dissolved oxygen, nitrate-nitrogen and phosphate-phosphorus) and control of blue-green algae. Up to the second week of culture, no difference in growth rate of pangasid catfish was observed among the three treatments. Growth rate was higher in T₂ from 10th week until the final harvest. The maximum input cost was required for T₁, while T₂ required least input cost followed by T₃. Economic analysis showed maximum profit in T₂ followed by T₃ and T₁. In composite culture system, pangasid catfish took only three and a half months to reach good marketable size and there was no off-flavour. Moreover the input cost came down to 50% by replacing 50% pangasid catfish by silver carp. In this way, three successive crops are possible in the same pond in a year. Poor farmers who are not able to invest much money will be benefited from the present study as they can get higher production of pangasid catfish and an extra crop of silver carp investing less money than pangasid catfish monoculture.

ECOLOGY AND MANAGEMENT OF EUGLENOPHYTES IN AQUACULTURE PONDS FOR IMPROVING FISH PRODUCTION

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As farmers are facing various problems with marked reductions in total fish production due to bloom of euglenophytes, experiments were conducted in on-campus research ponds of Bangladesh Agricultural University as well as in rural farmers' ponds to evaluate the ecology and management of euglenophytes in ponds for improving fish production. In our first ecological study, heavy blooms of euglenophytes were observed in the ponds where chicken manure was applied. The bloom of this group was influenced by a set of environmental factors such as comparatively higher water temperature, acidic pH and higher phosphate and nitrate concentrations. To control the euglenophytes bloom the second experiment with a set of four treatments each with three replications was conducted in 12 experimental ponds. In ponds of treatment 1 (T1) one third of the water surface was covered by duckweed (*Lemna minor*); in treatment 2 (T2) 0.5 kg lime/decimal/month was applied; in treatment 3 (T3) both duckweed as in T1 and lime as in T2 were applied; ponds of treatment 4 (T4) were considered as control where neither duckweed or lime was applied. The species composition and density of fishes including silver carp (*Hypophthalmichthys molitrix*) and silver barb (*Barbodes gonionotus*) which consumes euglenophytes were common in all treatments. The highest cell density of euglenophytes was found in the ponds of T4 followed by T1, T2 and T3. Acidic pH was found to be most conducive for the bloom of euglenophytes. In T1 ponds a thin bloom was observed where pH was neutral or slightly alkaline. In ponds T2 and T3, bloom of euglenophytes did not occur possibly due to alkaline pH and shade and nutrient absorption by duckweed. The grazing on euglenophytes by the silver carp and silver barb also had some contribution in controlling bloom though the species ratio and density of fishes were same in the ponds of all treatments. Growth of fishes was comparatively higher in the ponds of T1 and T3 which might be due to better water quality and availability of adequate food. The lower growth was recorded in the ponds of T4 which might be due to euglenophytes bloom. Thick euglenophytes bloom inhibited the light penetration which hampered photosynthesis of other plankton with causing lower dissolved oxygen. As a result, the growth of beneficial plankton as well as the final growth of fishes in the ponds of T4 decreased markedly. Gut content analysis revealed that silver barb and silver carp frequently consumed euglenophytes.

SHOREFLY'S LARVAE: A POTENTIAL BIOLOGICAL AGENT FOR DEGRADING ORGANIC IN SEDIMENT OF SHRIMP FARM

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One of the constraints for the success of marine shrimp farming is the proper management of the organic matter contamination in the pond sediment. It has been estimated that the rate of prawn wastes accumulated in ponds range between 100×10^3 kg/ha/yr and 300×10^3 kg/ha/yr (Boye, 1992; Smitch, 1993). This heavy organic matter loaded in ponds will lead to anaerobic condition and production of toxic gases such as hydrogen sulfide, nitrous oxide and methane. These gases are highly toxic to aquatic organisms especially to shrimps. This will lead to high mortality and retardation growth of shrimps and cause server lost of investment. Many techniques have been innovated for degrading organic matters contamination in sediment of prawn farms. However, they are expensive, not reliable, or environmental unfriendly. We manage to isolate and mass culture of marine shorefly larvae (*Hydrellia* sp) which are active in consumption of organic matters in shrimp pond sediment. In laboratory, the optimum conditions for degradation of organic in sediment are set at salinity 20.0 g L^{-1} , pH at 7.4, temperature at 40.20°C and sediment to water ratio at 1.00:2.37 with 100 4-days old larvae of *Hydrellia* sp /20 grams of sediment. It can remove 85% of organic in the sediment within 4 days under the optimal condition. This biological treatment technique can also be applied efficiently for the treatment of organic contamination in mangrove soils and in fish cake processing plant's wastes. At the end of the treatment, larvae are consumed by other organisms before they turn into adult shore flies. Therefore this biological technique is cheap, clean, efficient, and environmentally friendly in removing organic wastes in sediment.

**PROLIFERATION OF RICKETTSIA-LIKE ORGANISM PARASITIZING IN THE OYSTER
Crassostrea ariakensis GOULD, IN BALB/C MOUSE**

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The BALB/c mice were infected with Rickettsia-like organisms (RLO) purified from the gills of oyster, *Crassostrea ariakensis*. The BALB/c mice were divided into four groups for: injecting the RLOs; injecting the RLOs treated with penicillin; injecting the RLOs treated with penicillin and Cyclophosphomidum(CP) at the same time; (4): control group, injecting the sterilized PBS. The infection study involved three generations of the mice. The infected mice were anatomized at different times and RLOs were detected with direct staining technique, histological section and transmission electron microscope. RLOs were found in the spleens of the three generations of mice and were much more in 3rd group mice. The conclusion was: RLO is innocuous or low virulent to BALB/c mouse and is penicillin resistant; RLOs may propagate in the mouse bodies, but RLOs can propagate much more in the mouse that is inhibited the immunity function. In this study, the BALB/c mouse model for proliferation of Rickettsia-like organism parasitizing in *C. ariakensis* was successfully set up. The culture method of RLOs from marine mollusks in the terrestrial animal is significant for solving the animal model of RLO proliferation for further research on molecular immunology and molecular biology of RLO, and provides the pathological model for researching the pathogenic mechanism of RLO. This is the first report on the proliferation of Rickettsia-like organism from marine mollusks in terrestrial animal.

**DETECTION OF RICKETTSIALES-LIKE PROKARYOTE ASSOCIATED WITH
MORTALITY OF *Chlamys farreri* BY NESTED PCR**

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Mass mortalities have occurred in farmed *Chlamys farreri* since 1996 in China. An obligate intracellular rickettsiales-like prokaryote (RLP) was suggested as an agent. Partial 16S rRNA gene of 1319bp was amplified, cloned, sequenced, analyzed and identified by in situ hybridization. Phylogenetic analysis shows that the RLP is a new member of Rickettsiales. There are three segments unique to the RLP. To detect the RLP rapidly, sensitively and accurately, a nested PCR method was developed. Two pairs of primers were designed, based on the 16S rDNA, to specifically amplify a 203 bp segment of the RLP. Positive reaction was obtained for *C. farreri* samples from the diseased animals which were known to infect with the RLP by symptom, histology and microscopic examination while negative reaction was obtained for those collected from farms in which no disease has happened, and identified as RLP free by microscope. Results show that the method is sensitive and accurate. The establishment of effective detecting method is significant for diagnose and control of RLP disease in *C. farreri* in time.

**ESTABLISHMENT OF MOUSE MODEL OF A RICKETTSIALES-LIKE PROKARYOTE
(RLP) OF SCALLOPS *Chlamys farreri***

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The rickettsiales-like prokaryote (RLP) discovered in scallop, *Chlamys farreri* was suggested as the agent responsible for mass mortality of *Chlamys farreri* and was named as *Sinorickettsia chlamys*. To get stable, not enslaved to season and field, laboratory source of the RLP for the researches of immunology, molecular biology and pathogenesis at molecular level. The present paper tries to determine whether *S. chlamys* can infect mammals as a new animal model. Mice (Balb/c) were injected intra abdominally with purified RLP and sacrificed 11 days later to examine under microscope and use for nested PCR detection specific to RLP. Despite the groups only injected RLP in PBS, two parallel groups were injected RLPs and combined with cyclophosphamide and penicillin respectively. Control group was injected with PBS. With above same methods the homogenate of spleen of each group were injected into passaging groups and total of three passages were carried out continually each group. Results showed that the three experimental groups appear languish, hair shocking, twitching and anorexia symptoms at 5 days after injection. After dissection, spleens appeared tumescent. On the impressed slides of spleens and dried slides of blood drop, moderate numbers of bacteria with morphological features of the RLP in *C. farreri* were observed. Some bacteria inclusions were observed nearly filling the whole host cells and some bacteria were observed releasing from damaged macrophages. The infection of the group only injected with RLPs and the group injected RLPs with penicillin were basically equal, lighter than that of the group injected RLPs and the group injected RLPs with cyclophosphamide. Nested PCR with template extracted from the mice of all the groups of the three generations challenged with *S. chlamys* could get the specific segment. No symptom and RLP infection were observed in control group of each generation. No specific segment was amplified in nested PCR with template extracted from control group. In conclusion, *S. chlamys* can infect mouse Balb/c and reproduce, passage in it. Balb/c can be used as an animal model of *S. chlamys*. Cyclophosphamide can help the infection of *S. chlamys* to Balb/c slightly. Penicillin makes no impact on the infection of RLP to Balb/c. This is the first paper report the infection of mammal with RLP originated from marine cultured *C. farreri*.

PURIFICATION AND ANTIGENIC CHARACTERISTICS OF THE RICKETTSIA- LIKE ORGANISM FROM THE OYSTER *Crassostrea ariakensis* GOULD

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Rickettsia-like organisms are the etiological agent infecting the oyster, *Crassostrea ariakensis* heavily. Because of the lack of molluscan cell line for in vitro culture of specific intracellular prokaryotes, antigenic analysis of the rickettsia-like organism has been limited by the inherent difficulties of purifying them. In this report, use of different speed centrifugation and renografin density gradient centrifugation to purify the rickettsia-like organism directly from the infected tissues of the oyster, is described. The purity and integrity of purified prokaryotes were verified by electron microscopy. Specific polyclonal antiserum of the mouse was prepared for serological characterization of RLO. Thirteen major constitute proteins were electrophoretically identified, their molecular weights ranging between 99 and 17 KD, and the percentages of RLO proteins were analyzed by scanning apparatus. The serum above was used in an immunoblot of proteins from purified prokaryotes to identify 3 major antigens. The results from this study have furthered our knowledge of the protein antigens of RLO and identified several proteins potentially useful in diagnostic assays or for production of experimental vaccines.

AQUEOUS EXTRACT OF MATURE PODS OF *Moringa oleifera* ACTS AS AN IMMUNOMODULATING AGENT IN INDIAN MAJOR CARPS *Labeo rohita* (HAM.)

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Moringa oleifera is an important medicinal plant (Under the family - Moringace) and distributed throughout India. Hot water aqueous extract of the mature pods of *Moringa* was collected, centrifuged and the supernatant collected. Polysaccharides were collected from this supernatant by repeated precipitation in ethanol and the precipitate was lyophilized and used as a crude extract for this experiment.

The lyophilized extract of these pods as 0.01, 0.1 and 1.0 % were added in the experimental feed and fed @ 2% of their body weight per day for three weeks to young *Labeo rohita* (Ham). A separate group of *Labeo* was reared with the same formulated diet, devoid of pod extract. At the end of the experimental period, various non-specific defence mechanisms, including respiratory burst (NBT cell), phagocytic ratio (PR), phagocytic index (PI), serum protein level and albumin globulin ratio of the fish were measured.

Labeo rohita fed with 1.0 % lyophilized pod extract exhibit significance in non-specific immune response as compared to the control group after the three-weeks feeding trial.

This lyophilized extract showed significant macrophage activity through the release of nitric oxide on MOUSE monocyte cell line (J744.1).

Table 1 Estimation of phagocytic ratio (PR), phagocytic index (PI), & number of NBT positive cells

Treatment	PR	PI	NBT cells
Treated (1% Extract)	12	1.53	35
Control	7	1.21	12

DIPLECTANIDS FROM ORANGE-SPOTTED GROUPER *Epinephelus coioides* CULTURED ALONG THE WEST COAST OF THAILAND

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Orange-spotted grouper (*Epinephelus coioides*) from cage-cultured area in Krabi (West coast of Thailand) were examined for monogeneans. The fish of 4-5 inches were caught by fishermen and released into floating net cage and cultured to marketable size of 1-1.2 kg. Ten fish were sampled twice a month for a 10 month- study period. The fish samples were examined for external and internal parasites. Six species of diplectanid monogeneans were found viz., *Diplectanum grouperi*, *Pseudorhabdosynochus coioidesis*, *P. lantauensis*, *P. epinepheli* and another two new *Pseudorhabdosynochus* species (spp. A and B). Juveniles of diplectanids were also observed in some cases during the study period.

Diplectanum grouperi was the most dominant species, indicated by highest prevalence and mean intensity (Table) followed by *Pseudorhabdosynochus coioidesis*. There were no significant differences in term of abundance among their own groups during the sampling period. Overall water quality parameters, DO (4.6 -6.5 ppm), pH (7.1 - 7.9), temperature (27 - 31°C), salinity (20 – 32 ppt), unionized ammonia (0.0 – 0.017 ppm) remained stable during the study period, except for transparency (42.5 – 155.0 cm.) which was associated with the rainfall and tide. The lower degrees of prevalence and intensity of monogeneans might be due to the lower transparency. In most cases the fish showed no clinical signs of monogenean infection.

Table Mean intensity and prevalence of diplectanids

Diplectanid species	Prevalence (%)	Intensity	Mean intensity
<i>Diplectanum grouperi</i>	96	126	131
<i>Pseudorhabdosynochus coioidesis</i>	83	53	65
<i>Pseudorhabdosynochus epinepheli</i>	45	6	12
<i>Pseudorhabdosynochus lantauensis</i>	14	1	4
<i>Pseudorhabdosynochus</i> sp. n. A	89	36	40
<i>Pseudorhabdosynochus</i> sp. n. B	34	4	12
Juveniles of diplectanids	75	12	16

IMMUNOSTIMULATORY EFFECT OF MEDICINAL PLANT *Nyctanthes arbortristis* IN *Oreochromis mossambicus*

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An immunostimulant is a substance that elevates nonspecific defense mechanisms and specific immune response if followed by vaccination or infection. Though many synthetic and natural substances have been tested for their immunostimulating properties, traditional herbal medicine seems to be a rich source of active substances for immunostimulation. The objective of the present study was to investigate the effect of water-soluble and hexane-fractions of *Nyctanthes arbortristis*—leave on the immune responses of *Oreochromis mossambicus*. The fractions were administered intraperitoneally at doses of 3.2mg, 16mg, 80mg or 400mg Kg⁻¹ and assessed for specific immunity in terms of antibody response, nonspecific immune mechanisms in terms of lysozyme activity and intracellular reactive oxygen species production and integrated immunity in terms of relative percent survival (RPS). All the doses of both the fractions tested significantly enhanced primary and secondary antibody response to *Aeromonas hydrophila*. Similarly, the serum lysozyme level was also enhanced by all the doses of water soluble and hexane fractions tested. The doses of 16 mgkg⁻¹ and 400mgkg⁻¹ of hexane fraction enhanced the intracellular reactive oxygen species production whereas in case of water soluble fraction the doses of 3.2mgkg⁻¹ and 16mgkg⁻¹ enhanced the intracellular reactive oxygen species production. The hexane fraction gave better protection against experimental infection with *A.hydrophila* when compared to water soluble fraction.

Table 1 Table showing the effect of water soluble & hexane fractions on the immune responses of *Oreochromis mossambicus*

Dose tested Kg ⁻¹	Primary &Secondary Antibody response (Log ₂ titre)		Serum lysozyme activity (Units/ml)		Intracellular reactive oxygen species (OD)		Disease resistance (RPS)	
	WSF (Day 14)	HF (Day 14)	WSF (Day 10)	HF (Day 10)	WSF (Day 2)	HF (Day 8)	% expt. mortality	% cont. mortality
0 mg	6±0	7±0	167.5±18.2	164.1±9.96	0.46±0.06	0.33±0.07	-	-
3.2 mg	8.6±0.33*	9.5±0.34*	267.5±27.0*	374.1±22.7*	0.7±0.07*	0.48±0.04	27	29
16 mg	8.1±0.13*	10.3±0.21*	205±18.18*	385.8±30.8*	0.8±0.06*	0.7±0.03*	19	37
80 mg	9±0.26*	8.5±0.22*	254±10.63*	330.8±37.2*	0.27±0.05	0.39±0.04	42	54
400mg	8±0*	9.5±0.43*	232.5±21.5*	435.8±48.8*	0.23±0.03	0.6±0.05*	50	67

* P<0.05 when compared with control

IMMUNOSTIMULATORY EFFECT OF SELENIUM IN TILAPIA

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Fish diseases have been traditionally treated using chemicals. Selenium is a well-known essential nutritional trace element for animals and man. It plays an important role in the catalytic activity of many enzymes. Unlike most of the metals, Selenium generally potentiates the immune response rather than suppressing it. In the present study, the modulatory effect of sublethal doses of selenium on the antibody response to bovine serum albumin (BSA) and sheep red blood corpuscles (SRBC) using passive haemagglutination and direct haemagglutination assays respectively, the total and differential blood leucocytes count and the disease resistance against *Aeromonas hydrophila* in *Oreochromis mossambicus* was investigated.

A significant enhancement ($P < 0.05$) in antibody response was observed on the peak day (day 10) for all the doses except 0.1% LD₅₀ of selenium tested. A similar enhancement of the total count of peripheral blood leucocytes was noted. The disease resistance of selenium treated *O. mossambicus* was stimulated when they were challenged with virulent *A. hydrophila*. These findings show a general enhancement of immunity in *O. mossambicus* which will possibly help the fish defend against pathogens. This study may help in developing an alternative strategy to manage the outbreak of diseases in aquaculture.

Table 1 Effect of Selenium on the antibody response (peak day) of *O.mossambicus* to BSA/SRBC.

Antigen	% LD ₅₀ of Selenium administered				
	0	0.01	0.1	1	10
BSA	8 ± 0.6	9.3 ± 0.4*	8.9 ± 0.4	9.5 ± 0.2*	9.2 ± 0.3*
SRBC	4.8 ± 0.4	6.5 ± 0.5*	6 ± 0.6*	6 ± 1*	6.8 ± 0.4*

Table 2 Effect of Selenium on the peripheral blood leucocytes (peak day) in *O.mossambicus*.

Peripheral Blood Leucocytes	% LD ₅₀ of Selenium administered				
	0	0.01	0.1	1	10
Total Count (X10 ³ /mm ³)	13.5 ± 1.9	19 ± 1.5*	17.5 ± 1.3*	17.3 ± 1.6*	15.5 ± 1.7*
Differential Count (%)	L	70 ± 1.7	76 ± 1.7*	78 ± 0.9*	76 ± 1.7*
	G	28 ± 1.4	24 ± 1.2*	21 ± 0.8*	23 ± 1.6*
	M	1 ± 1.6	0 ± 1.2	1 ± 1.3	1 ± 1.1

L-Lymphocytes G-Granulocytes M-Monocytes

Table 3 Percentage mortality of selenium-administered *O.mossambicus* challenged with *Aeromonas hydrophila*

Route of Metal Administration	% LD ₅₀ of Selenium administered				
	0	0.01	0.1	1	10
Intraperitoneal	80	36.7	40	56.7	80
Exposure	71	27	10	10	23

* $P < 0.05$ when compared with control

STREPTOCOCCOSIS IN *Lates calcarifer*

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Streptococcus sp. isolated from infected Seabass (*Lates calcarifer*) can grow on medium at pH 9 and salinity 0 ppt. The lethal dose where the mortality is 50 percent (LD₅₀) was 1.937 X 10² colony forming unit (injected intraperitoneally) after 14 days of infection. Diseased fish showed dark body coloration, erratic swimming, exophthalmia, dropsy, pale liver, splenomegaly and hemorrhage in the brain. Blood parameters i.e. haematocrit, haemoglobin, plasma protein and red blood cell of infected fish were decreased, while white blood cells were increased. Histopathological changes in diseased seabass were necrosis, vacuolization and granuloma of the liver, increased the number of melanomacrophage in liver and spleen, shrinkage of glomerulus in the kidneys, inflammation and necrosis of the spleen and heart, hemorrhage in brain, hyperplasia and telangiectatic of the gill while vacuolization and capsulation of the exophthalmic eyes were observed.

**EFFECT OF *Cinnamomum cassia* OIL EXTRACT ON STREPTOCOCCOSIS IN
Oreochromis niloticus X *O. mossambica***

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Streptococcosis caused by *Streptococcus* spp. was found in cultured sex reversed red tilapia (*Oreochromis niloticus* × *O. mossambica*). The disease signs were lethargic and spiral swimming, ascites, cataract and exophthalmia, hemorrhages on the skin, fins and brain. Lethal dose (LD₅₀) of these pathogenic bacteria was 1.46x10² colony forming unit (injected intraperitoneally). Cinnamon bark oil extract showed minimal inhibitory concentration of 250 ppm against pathogenic *Streptococcus* spp. The results from gas chromatography and mass spectrophotometer showed that the extract contained 3 active substances e.g. trans-cinnamaldehyde (C₉H₈O) 83.08%, coumarin (C₉H₆O₂) 12.60% and cinnamic acid (C₉H₈O₂) 2.15%.

Fish fed diet supplemented with 250 ppm cinnamon oil extract exhibited higher relative percentage survival than those fed diet supplemented with antibiotic (Sulfamethoxazole) and high concentration of cinnamon oil extract (500 and 1,000 ppm) after challenged with *Streptococcus* spp.

TRYPANOSOMIASIS IN *Clarias macrocephalus* X *C. gariepinus* IN THAILAND

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Infestation of *Trypanosoma* sp. (Kinetoplastida) (Figure 1) in hybrid catfish (*Clarias macrocephalus* X *C. gariepinus*) and some freshwater fishes was studied showing such parasitic infestation was specific only in catfish. The virulence as determined by LD₅₀ for 5 days was 2.28×10^{10} cells in a fish sample. The parasitic infestation caused hematological changes by significant reduction of red and white blood cells. Similar trends were noted for hemoglobin and hematocrit which decreased significantly ($p < 0.05$) in the infected fish. The reverse trend was recognized for serum protein and leukocrit which increased in the infected samples. The density gradient centrifugation technique using 50% Percoll in 0.85% NaCl solution was employed in the isolation of parasites. The study of antibody levels in serum showed that the infected hybrid catfish could develop the antibody which reached its peak level at 14 days after infection. Mild inflammation of the gills, liver, kidney, spleen, stomach and intestine was observed by histological studied. As well as the presence of parasites in the blood streams and sinuses were noted. Marked reductions were recorded for mature red blood cells whilst the formation of immature red blood and phagocytic cells were at higher rates as compared to healthy individual.

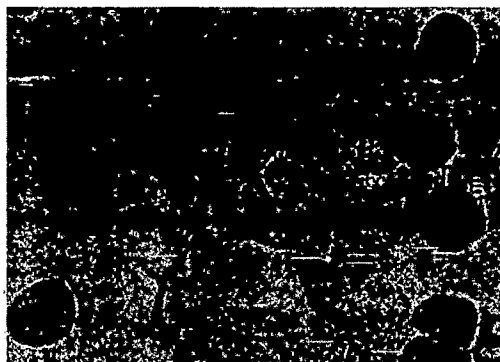


Figure 1 Characteristics of *Trypanosoma* sp. (Kinetoplastida) in hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) (p = parasites; rbc = red blood cell) (Geimsa; bar = 10 μ m)

PATHOGENICITY OF *Edwardsiella tarda* IN MARINE FISH SPECIES; JAPANESE FLOUNDER, RED SEA BREAM AND YELLOWTAIL

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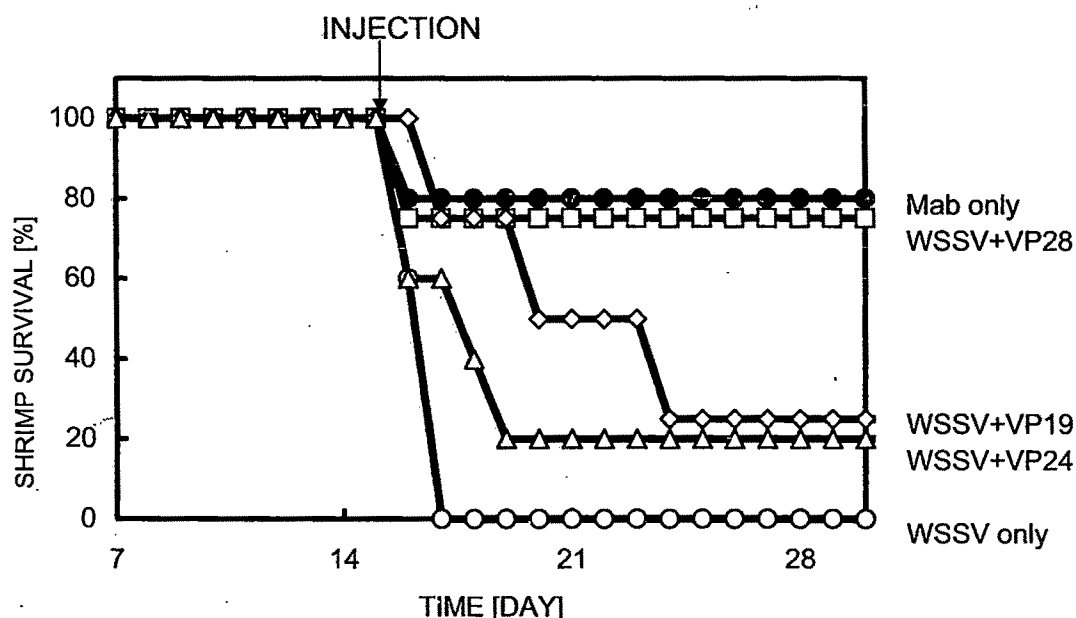
In Japan, edwardsiellosis caused by *Edwardsiella tarda* is an economical threat to freshwater and marine fish culture; particularly in Japanese eel (*Anguilla japonica*), Japanese flounder (*Paralichthys olivaceus*) and red sea bream (*Pagrus major*) culture. Two phenotypes, typical and atypical *E. tarda*, have been reported from diseased fish. Characteristics of typical strains are motile and do not produce acid from arabinose and mannitol. Typical strains are mainly isolated from freshwater fishes and Japanese flounder. Strains isolated from diseased red sea bream and yellowtail (*Seriola quinqueradiata*) is identified as atypical *E. tarda*. However, difference in pathogenicity between typical and atypical strains has not been compared. We examined the pathogenicity of both phenotypes against marine fish species; Japanese flounder, red sea bream and yellowtail.

Five typical strains isolated from eel, tilapia or Japanese flounder and two atypical strains isolated from red sea bream were used in this study. To test the susceptibility, each fish group was injected intraperitoneally with 10^5 - 10^6 cfu/fish of bacteria suspended in sterilized phosphate buffered saline. Japanese flounder and yellowtail were sensitive to all strains, and they began to die 3 to 5 days or 1 to 2 days after inoculation, respectively. In contrast, red sea bream mortality occurred only when inoculated with the atypical strain. They began to die 1 or 2 days after injection and all fishes were dead within 6 days. This study indicates that there is a difference in pathogenicity between the typical and atypical strains.

APPLICATION OF MONOCLONAL ANTIBODIES FOR DETECTION OF WHITE-SPOT SYNDROME VIRUS AND NEUTRALIZATION OF ITS INFECTION

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White spot syndrome virus (WSSV) is a major viral disease agent of penaeid shrimp. A number of studies have been done for basic research and development of a diagnostic kit for WSSV. One of the promising diagnostic tools to detect viral pathogens in shrimp farms must be the immunological methods using monoclonal antibody because it doesn't need any expensive equipment and time for the analysis can be very short. However, it was found that WSSV is a virus encapsulated with envelope proteins which are very fragile and easily destroyed by ordinary virus purification procedure. Since purification of antigen is an essential step for a manipulation of monoclonal antibody, purification method for WSSV was modified to obtain WSSV as virion. After inject of virion purified by modified method, more than 50 hybridoma clones producing antibodies which have binding capacity with different viral protein were obtained. Neutralization effect of each monoclonal antibody on WSSV infection was investigated and it was found that monoclonal antibody against 28kDa had the highest neutralization effect, suggesting that 28kDa viral protein may play a significant role on WSSV infection.



Shrimp survival after injection of WSSV together with monoclonal antibodies specific to different kinds of viral proteins

**EFFICACY OF OUTER MEMBRANE PROTEIN (OMP), INACTIVATED WHOLE CELLS
AND HAEMOLYSIN VACCINATION IN *Tilapia mossambicus* AGAINST *Aeromonas
hydrophila***

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Fingerlings of *Tilapia mossambicus* were immunized intramuscularly with outer membrane protein (OMP) (150 µg/fish), heat inactivated whole cells (0.1ml of 10⁶ cells/ml) and haemolysin (0.5 ml). OMP and Haemolysin were prepared following the modified method of Kabir (1980) and Asao et. al (1986) respectively. Fishes immunized with OMP were challenged with *Aeromonas hydrophila* (0.1ml of 10⁶ cells/ml) after 1, 4, 8 and 16 days of post vaccination. Similarly fishes immunized with formalin, and heat killed *A. hydrophila* and heat inactivated haemolysin were also challenged with *A. hydrophila* after 3days post vaccination. Maximum survival was observed in OMP vaccinated fish challenged with *A. hydrophila* after 8 days of post vaccination (90%) (Table). In the group fish immunized with inactivated whole cells and haemolysin, mortality was reduced to 20 -30 % when compared to control (90 % of the fish died when challenged with *A. hydrophila*). Injection of OMP enhances the cell mediated response (Lysozyme, Neutrophil activity and WBC) in tilapia.

Table Relative Percentage Survival of *Tilapia mossambicus* after OMP, Inactivated *A.hydrophila* vaccination.

Groups	Relative Percentage Survival
Control (<i>A. hydrophila</i>)	0
OMP + <i>A. hydrophila</i>	0
OMP + <i>A. hydrophila</i> (injected 1 day PV)	0
OMP + <i>A. hydrophila</i> (injected 4 days PV)	0
OMP + <i>A. hydrophila</i> (injected 8 days PV)	70
OMP + <i>A. hydrophila</i> (injected 16 days PV)	80
OMP alone	90
Formalin killed <i>A. hydrophila</i> *	70
Heat inactivated <i>A. hydrophila</i> *	70
Heat inactivated haemolysin*	80

PV – Post vaccination

* Fishes were challenged with *A. hydrophila* after 3 days PV

PHYLOGENETIC ANALYSIS OF RED SEA BREAM IRIDOVIRUS – LIKE VIRUSES IN SOUTHEAST ASIA

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Red sea bream iridovirus (RSIV) causes one of the most serious viral disease of farmed marine fish species in Japan and similar diseases have been observed not only among marine fishes but also in fresh water ornamental fishes in Southeast Asia. However, the causative agents have not been well characterized genetically until recently except for a few cases. In this study, full-length major capsid protein (MCP) gene and adenosine triphosphatase (ATPase) gene, both of which are considered to be suitable targets for genetic analysis, were identified from 14 viral samples from groupers (*Epinephelus* spp.), mullet (*Mugil cephalus*), sea bass (*Lateolabrax japonicus*), dwarf gourami (*Colisa lalia*) and other fish species in Singapore, Philippines, Malaysia and Hong Kong to compare with their counterparts of RSIV, closely related infectious spleen and kidney necrosis virus (ISKNV) and other related viruses belonging to fifth genus of family Iridoviridae. In order to achieve this purpose, PCR amplifications using degenerated primer sets for both RSIV and ISKNV were adopted. Extracted DNA from supernatant of cultured viruses or those from affected fish organs were used as templates. Resultant amplicons were TA-cloned into plasmid vector, and the three clones for each were sequenced respectively and consensus nucleotide sequences were determined. Genetic correlation of these Southeast Asian RSIV-like viruses, RSIV, ISKNV and other related viruses deposited to DNA databank were investigated by phylogenetic analysis based on nucleotide sequence or deduced amino acid sequence of MCP and ATPase genes. The results demonstrated that these Southeast Asian RSIV-like viruses were divided into two clusters, one of which includes RSIV and another includes ISKNV. Specifically, the viruses of groupers were divided into two groups. Grouper iridovirus (GIV), which belongs to genus Ranavirus, also has been reported from Southeast Asia. Therefore, iridoviruses of groupers in Southeast Asia can be classified into at least three kinds of viruses.

PCR BASED DETECTION OF *Aeromonas hydrophila* IN *Cyprinus carpio*

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Aeromonas hydrophila causes hemorrhagic septicemia in fishes, reptiles and amphibians under stress conditions. The present study describes the polymerase chain reaction (PCR)-based assay for detection of the *A. hydrophila* in the diseased fishes and water isolates. Two oligonucleotide primers of 22 and 21 bases respectively were designed to specifically detect *A. hydrophila* by PCR assay. For the primer design, a software program GeneTool iite version 1.0, a BioTool product (www.BioTools.com) and www.DoubleTwist.com and Mac Vector programs were used. From the nucleotide sequences of the five selected haemolysin gene containing bacteria (*A. hydrophila* (strain 28SA) (X65044), *A. hydrophila* (strain AH1) (X65045), *A. sobria* (X65046), *A. caviae* (U40711) and *A. hydrophila* (AB021152).), obtained from GenBank database, the ORF's were obtained and Pairwise identity percentage was observed after aligning the selected ORF's. For the conserved regions, primers were designed using the program Mac Vector. A DNA fragment of 683 bp was amplified from the sources of standard MTCC strains as well as the field isolates (identified by biochemical methods). The PCR fragment was checked by digestion with the restriction enzyme *Pst*. I. The sensitivity of the PCR assay was determined to be 1 pg of DNA. Primer specificity was checked by comparison using Blast search as well as by PCR assays. Detection of *A. hydrophila* in infected samples by PCR amplification proved to be highly sensitive and specific. The designed Oligonucleotide primers failed to detect other bacteria *Vibrio cholerae*, *Aeromonas salmonicida*, *Aeromonas sorbida* and *Pseudomonas aeruginosa*. This supports the specificity of PCR assay for *A. hydrophila* detection in tissue and water samples.

ADVANCED MOLECULAR DIAGNOSTICS FOR THE RED SEABREAM IRIDOVIRAL DISEASE

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Systemic iridoviral infection in fish has been reported for a diverse number of species over a wide geographical range. The red seabream iridovirus (RSIV) is the causative agent of the red seabream iridoviral disease (RSIVD), an infectious disease affecting cultured marine fishes in Japan during the summer season. The virus was first isolated in diseased red seabream *Pagrus major* in western Japan in 1990 and has since infected 31 marine fishes spanning three taxonomic orders. Recently, similar outbreaks of the viral disease have been observed in some Asian countries like Korea, Hong Kong, Singapore, Taiwan and Thailand.

For effective management in the control of this viral disease before the onset of massive mortality, several detection methods have been developed to ascertain the presence of the virus in the cultured stock. These include cell culture, Giemsa staining, immunofluorescence using monoclonal antibody and conventional PCR. However, these methods possess limitations which hamper their large-scale application. The use of histological and biochemical methods may sometimes fail to detect the viral antigen during the early stages of infection. On the other hand, conventional PCR sometimes result to insufficient specificity and low amplification efficiency.

To overcome these limitations in viral diagnosis, we developed the quantitative real-time PCR and the loop-mediated amplification (LAMP) reaction for the rapid detection of RSIV. Quantitative real-time PCR has gained wide acceptance as a diagnostic tool because it makes possible to detect and quantify the virus antigen without the need for cell culture. LAMP on the other hand, is a relatively new technique that allows amplification of the target DNA within a short span of time (1 hr) at isothermal conditions (60-65°C). It requires four primers which are based on six distinct regions of the target DNA; hence a high degree of specificity is obtained. The presence or absence of the target DNA in the sample is judged by the appearance of a white precipitate, magnesium pyrophosphate.

The LAMP and real-time PCR methods that we have developed for RSIV diagnosis were at least 10 and 100 times more sensitive than conventional PCR, respectively. Both methods were specific for RSIV since no amplification reactions were detected in unrelated viruses and in apparently healthy fish. Using real-time PCR, we were able to quantitate the viral load in dead fish as well as the amount of viral antigen in the target sites of RSIV in the infected fish. After viral challenge, the virus was detected in the survivors which otherwise tested negative by conventional PCR. For the LAMP reaction, we found high correlation between the amount of input virus DNA and turbidity at the end of the reaction; hence it may have the potential to quantify the amount of virus in the infected fish.

With the advanced molecular diagnostics that we have developed for RSIV, alternative options are being provided to the aquaculture industry for a rapid, accurate as well as possibly quantifying virus infection in fish before the onset of heavy mortality leading to tremendous economic losses. These techniques may also be applied for the detection of other viruses affecting cultured fish and shellfish.

DEVELOPMENT OF DNA VACCINES FOR JAPANESE FLOUNDER *Paralichthys olivaceus* AND ANALYSIS OF THE IMMUNE RESPONSE AFTER VACCINATION

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Japanese flounder *Paralichthys olivaceus* is one of the major aquaculture species in Japan. However, culture intensification for this species is hampered by problems associated by both biotic and abiotic factors. Disease problems due to either bacterial or viral pathogens have caused significant damage to the aquaculture industry because they affect the various life stages of the fish.

DNA vaccine technique for fish has been explored a decade ago. Earlier studies on DNA vaccination trials in fish, however, have used freshwater species as a model to elucidate the efficacy of DNA vaccination in fish. Here, we tried to establish the effectiveness of DNA vaccination in a marine fish species using two rhabdoviruses, the hiramé rhabdovirus (HIRRV) and the viral haemorrhagic septicaemia virus (VHSV). Furthermore, to better understand the immune response in vaccinated fish, microarray analysis was conducted.

The HIRRV glycoprotein (G protein) gene, HIRRV nucleocapsid protein (N protein) gene and VHSV Gprotein gene were cloned into mammalian expression plasmid vector under the control of the CMV promoter. These plasmid vectors were designated pHRV-G, pHRV-N and pCMV-VHSG, respectively. Japanese flounder juvenile of approximately 3 g in body weight were intramuscularly injected with 50 µl of each plasmid DNA solution in phosphate buffered saline (PBS). Kidney tissues were taken from five fish in each group at 1, 3 and 7 days post-immunization and stored at -80 °C until total RNA extraction for the microarray analysis. One month after vaccination, fish were challenged with live HIRRV (4.0×10^3 TCID₅₀ HIRRV/ fish) by the intramuscular route or with VHSV (1.0×10^3 TCID₅₀ VHSV/ fish) intraperitoneally. The cDNA microarray chip used in this study contained about 800 ESTs clones. Each gene was spotted in duplicate to facilitate comparison during the analysis.

Relative percentage survival of the fish injected with pHRV-G and pHRV-N was 86.5 and 1.5%, respectively upon HIRRV challenge. On the other hand, pCMV-VHSG immunized fish were completely protected. Microarray analysis showed the up-regulation of antiviral genes in pHRV-G and pCMV-VHSG immunized fish like Mx, ubiquitin-like protein and interferon-inducible 56 kDa protein until 7 days post-vaccination, while the pHRV-N lacked the ability to induce the expression of antiviral genes.

The result of this study suggested the possibility of utilizing the DNA vaccination technique not only in freshwater fish but also in marine fish if the G-protein gene is contained in the DNA vaccine. On the other hand, the N-protein gene did not confer strong protective ability to the host. Microarray analysis confirmed up-regulation of important immune-related genes after vaccination and thus leading to better protection in fish upon viral infection.

KINETIC TRANSCRIPTION PROFILE OF RED SEA BREAM IRIDOVIRUS AS CHARACTERIZED BY DNA MICROARRAY

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Red sea bream Iridovirus (RSIV), a piscine dsDNA virus from the family *Iridoviridae*, has been isolated as the causative agent of the red sea bream iridoviral disease in at least 31 marine fish species. To better understand the viral replication strategies and to get insights into the pathogenetic mechanism of the virus, we developed a viral DNA microarray containing 92 putative open reading frames (ORFs) of RSIV and established RSIV infection in a permissive cell culture system for time-course sampling. Total RNA was extracted from Japanese flounder natural embryo (HINAE) cells that had been infected or mock-infected with RSIV at 3h, 8h, 18h, 24h and 48h post infection (p.i). The total RNA was reverse transcribed to cDNA, labeled with fluorescence Cy5- or Cy3-dUTP and hybridized onto the microarrays.

The microarray analysis showed that the transcriptional profile of RSIV changed as it progressed through its life cycle. Viral genes started to be transcribed as early as 3h p.i and a rapid escalation of gene expression could be seen from around 8h p.i onwards. The DNA replication process was predicted to begin at approximately 8h after the infection in infected cells. The RSIV transcripts were also classified into 3 main temporal kinetic classes (Immediate-Early (IE), Early (E) and Late (L) gene class) based on treatments with cycloheximide (CHX) and phosphonoacetic acid (PAA), which act as *de novo* protein synthesis and viral DNA replication inhibitors, respectively. The microarray analysis identified 9 transcripts with IE expression kinetics, 40 transcripts with E expression kinetics and 38 transcripts with L expression kinetics. In summary, this viral DNA microarray study provided the first comprehensive analysis of the temporal kinetic transcription profile for RSIV and this technology may be applied to other piscine viruses.

***Vibrio alginolyticus* ASSOCIATED TO MASS MORTALITY OF ABALONE *Haliotis discus hannai* IN KOREA: SPECIFIC DNA DETECTION BY PCR**

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Vibrio alginolyticus strain PD2 originally isolated from cultured abalone in Ulsan, Korea in June 2002 was used in this study. The strain was isolated from the haemolymph of diseased abalones (*Haliotis discus hannai*) using tryptic soy agar. Abalone tissues fixed in Davidson's solution and separate sections were stained with H&E for bright field light microscopy examination. DNA extracted from axenic cultures using High Pure PCR Template Preparation Kit as outlined in the manufacture's protocol. The PCR primers used for amplification of DNA fragments spanning the 16S and 16S-23S intergenic spacers (IGSs) were targeted at highly conserved regions of the *Escherichia coli* 16S ribosomal RNA gene. The 16S-23S IGS sequence from this study has been deposited in the GeneBank database (Accession number, AY601670). Abalones, *Haliotis discus hannai*, originated from NFRDI (Busan, Korea) and measured between 3-4 g. A volume of 100 µl of bacterial suspension (4×10^4 , 10^6 CFU) was inoculated by injection into the right side of mantle. Sterile PBS was injected into the controls. Mortality of abalones was recorded daily for 1 week post-injection. Re-isolation and identification of the bacteria from the haemolymph of moribund abalone post-bacterial challenge was carried out using TSA (1% sodium chloride) and TCBS agar plates.

Bacteria isolated from the haemolymph of diseased abalones were Gram-negative, straight rod, swarming on TSA (+1% sodium chloride). On the basis of the results from the Biolog GN system and API 20E kit, the PD2 strain was identified as *Vibrio alginolyticus*. Four abalones were analysed by histology. Different ciliates were observed in abalone gills but no lesion was associated with their presence. Metazoan was found along the foot and sometimes, necrosis was noted in the around the metazoan. Bacteria were observed in the connective and muscular tissue of the foot and mantle. The bacteria infection was present in the four abalones examined and was believed to be systemic. Necrosis of the connective tissue and muscular fibres was noted and could be associated with the presence of the bacteria. Mortality of abalone with different bacterial concentration was noted. After injection of 10^6 CFU /ml of *V. alginolyticus* strain PD2, all abalone died within 1 day. Mortalities of abalones differed with bacterial concentrations. No mortality was observed in the control treatment. The bacteria were re-isolated from haemolymph of moribund experimental abalone and were reconfirmed to be the same species as that injected. PCR results were done for 16S and 23S rRNA PCR. The 1362-bp fragment and 880-bp were obtained from 16S and 23S rRNA PCR, respectively, and were subsequently sequenced. The alignment of 16S rRNA of *V. alginolyticus* strain PD2 with homologous sequence of *Vibrio* showed that the sequence of *V. alginolyticus* strain PD2 is identical to those sequences of *Vibrio*.

OIE WHITE SPOT SYNDROME VIRUS PCR GIVES FALSE-POSITIVE RESULTS IN *Cherax quadricarinatus*

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White spot syndrome virus (WSSV) is an intranuclear bacilliform virus (IBV) that is a serious, notifiable crustacean pathogen. WSSV has been responsible for worldwide mortalities in the penaeid aquaculture industry. The Office International des Epizooties (OIE) polymerase chain reaction (PCR) protocol for WSSV uses primer sets initially developed by Lo et al. (1996). These primers yield a first-step PCR amplicon of 1441 bp and a nested PCR amplicon of 941 bp. An amplicon (941 bp) purported to specifically detect WSSV was obtained when using template DNA extracted from *Cherax quadricarinatus* in a WSSV PCR detection protocol recommended by the OIE. Sequencing and analysis of the 941 bp amplicon and an occasional 550 bp amplicon from *C. quadricarinatus* revealed no phylogenetic relationship with WSSV, and suggested a possible lack of sufficient primer specificity for WSSV in the OIE test. This suggestion was supported by the fact that the OIE outer primer sequence (146F1) was present in both the forward and reverse position of the 941 bp nested amplicons from *C. quadricarinatus*.

This is the first time false-positive results have been confirmed using WSSV primers designed by Lo et al. (1996). In Australia in late 2000, putative WSSV infections were detected in crabs within the Darwin Aquaculture Centre and prawns within the aquaculture facilities of the Northern Territory University using the OIE protocol. The putative infections were thought to have arisen from the inadvertent use of imported green prawns as feed. Despite the lack of clinical disease, the positive PCR findings resulted in immediate slaughter of all crustaceans at both facilities and the Consultative Committee on Emergency Animal Diseases (CCEAD) agreed to conduct a survey of wild crustaceans (prawns and crabs only) to determine whether WSSV was present in Australia. The sampling strategy and testing methods used conformed to published OIE protocols. Positive WSSV results were obtained using the OIE PCR method at a further 3 sites but parallel samples assessed by bioassay, histology and *in situ* hybridisation all gave negative results.

The potential for false-positive PCR results emphasises that detection should always be confirmed by another diagnostic method. As WSSV is a notifiable pathogen, the consequences of false positive results are tough in WSSV free zones, and can lead to incorrect quarantine and unnecessary destruction of animals. Therefore urgent attention and revision is necessary for the current OIE PCR protocol for WSSV detection.

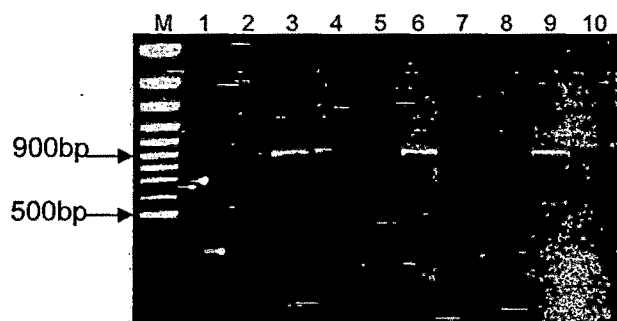


Figure 1 Inner nested PCR reaction using OIE WSSV primers. Three independent replicates of three samples produced the expected band of 941bp.
M = Marker, Lanes 1-9 = Samples, Lane 10 = Negative control.

**PROTEIN COMPOSITION OF *Cryptocaryon irritans*, A CILIATED MARINE FISH
PARASITE ISOLATED FROM SEABASS, *Lates calcarifer***

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Protein composition of *Cryptocaryon irritans* was determined by using one-dimensional polyacrylamide gel electrophoresis. Results showed 2 to 3 bands from membrane protein extracted from *C. irritans* and 7 bands from the mucus isolated from infected fish with or without trophont of *C. irritans*. Proteins with molecular weight of 15, 50-60 and 70kD were obtained from the encystment stage and 15kD from the T and Th. Mucus from infected fish with or without trophonts had proteins of molecular weight at 15, 25-30, 40, 40-50, 60, 60-70 and 70kD. Under silver staining, protein gel electrophoresis of the extracted membrane protein from the encystment stage showed a total of 3 bands namely: 15, 25-30 and 40-50kD compared to 2 bands (40-50 and 15kD) in protein extraction of T and Th while no band was found on the extracted protein from cyst wall of the encystment stage. The proteins of 15, 25 and 40-59kD on SDS-Page gel belonged to the *C. irritans* where 25kD was associated with the encystment stage. Other proteins of 25-30, 40, 60, 60-70 and 70kD belonged to the fish mucus.

STUDY OF VHS1 BACTERIOPHAGE LYSOGENY IN *Vibrio harveyi*

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Exposure of a bacterial suspension to a temperate bacteriophage will result in lysis of many cells and lysogenic conversion of some that contain a prophage plasmid or prophage element integrated into the host bacterial chromosome. The result is a bacterial clone of variable stability that may express genes from the acquired prophage. In this study, the lysogenic bacteriophage VHS1 produced very few, relatively unstable lysogenic clones of *V. harveyi*. These unstable clones produced both infected (lysogenic) and uninfected (non-lysogenic) progeny clones in the same culture. The uninfected clones were very stable and showed no lytic plaques when lawns were challenged with VHS1. However, they did show turbid plaques in dot plaque assays. Thus, they differed phenotypically from the original parental isolate, suggesting that interaction with VHS1 resulted in permanent genetic change. Despite this, the clones failed to show evidence of genetic material from VHS1 by southern blot hybridization and PCR assay, suggesting that the change had occurred in the host genome. Such clones may be called pseudolysogenic since they show some phenotypic characteristics of lysogenic clones but do not contain phage genes. This phenomenon has not been previously reported and the mechanism for it is unknown. Experiments revealed that *V. harveyi* clones lysogenized by VHS1 carried the prophage as an episome that did not integrate into the host chromosome. The VHS1 prophage could be detected by both southern blot hybridization and by polymerase chain reaction (PCR) assay. This situation explained the rapid loss of virulence of lysogenic isolates on continuous subculture in the laboratory.

CHARACTERIZATION OF Y-ORGAN CELLS IN THE PENAEID SHRIMP *Penaeus monodon*

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Growth in crustaceans is related to molting that is, in turn, regulated by at least two hormones, molt-inhibiting hormone (MIH) and ecdysone. The first one is synthesized in the X-organ and the latter in the Y-organ. The purpose of this study was to define the location of Y-organ in the black tiger shrimp *Penaeus monodon* by light microscopy (LM) and to examine the morphology and ultrastructure of Y-organ cells by transmission electron microscopy (TEM). For LM, the cephalothoraxes of juvenile *P. monodon* were perfused with Davison's fixative and processed through paraffin for hematoxylin and eosin (H&E) staining of tissue sections. For TEM, tissue regions presumed contain the Y-organ were removed from dorso-medial portions of the gill-branchial chamber, dissected into pieces, fixed with modified Millonig's fixative overnight at 4°C, and finally embedded in araldite resin. Semi-thin sections were stained with toluidine blue for LM and thin sections were stained with uranyl acetate and lead citrate for TEM. The Y-organ was divided into dorso-ventral and antero-posterior parts. The antero-posterior part was located dorsomedially at the boundary of the gill-branchial chamber as a thin sheath of tissue aligned along the chamber in an antero-posterior direction. Glandular cells were arranged in lobules with intervening hemal sinuses. The oval dorso-ventral part was located forward, ventral to the carapace adductor muscle. These organs were covered by layers of cuticle at the apical border without an intervening epidermal lining. Both parts of the Y-organ contained cells that were homogeneously spindle shape with long processes attached to the cuticle. By TEM, three types of cells were observed in different specimens. The first type showed prominently heterochromatic nuclei, numerous tubular mitochondria, and cytoplasmic vesicles. The second type possessed abundant ribosomes and vesicles and a moderate amount of mitochondria and vacuoles (i.e., a different proportioning of organelles than in the first type). Electron dense granules were packed inside some vesicles and myofilament-like structures were seldom found. The third cell type showed distinctive euchromatic nuclei with scanty cytoplasm and fewer organelles. Variation in the ultrastructure of Y-organ cells from specimen to specimen may be related to molt stages. The first and second TEM cell types may represent those found at the early and late stages of premolt, respectively, when the rate of ecdysone synthesis and secretion is high, while the third type may represent cells at the intermolt stage.

**CLONING, EXPRESSION AND PRODUCTION OF POLYCLONAL ANTIBODIES
AGAINST RECOMBINANT VP1 CAPSID PROTEIN OF TAURA SYNDROME VIRUS (TSV)**

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VP1 capsid protein encoding gene of taura syndrome virus was cloned into a pGEX-6P-1 expression vector and transformed into *E. coli*. After induction, the bacteria were able to produce the recombinant VP1 protein (rVP1). The recombinant protein was purified by SDS-PAGE and used for immunization in Swiss mice for polyclonal antibody production. The antibodies can be used to detect TSV infection in tissue by means of immunohistochemistry. In the future, monoclonal antibodies specific to rVP1 will be generated in order to develop of a simple immuno-based test kit for TSV detection with high accuracy and sensitivity at low cost.

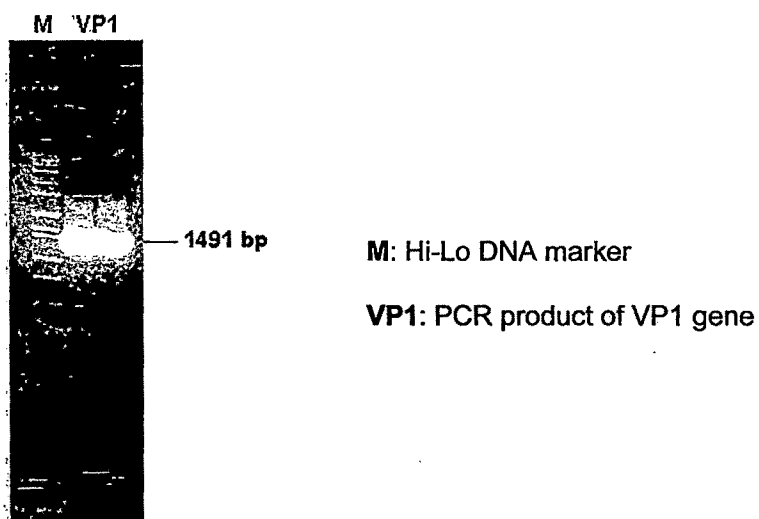


Figure 1 Ethidium bromide stained gel of VP1 PCR product

DEVELOPMENT OF SIMPLE IMMUNOBASED TEST KIT FOR YELLOW HEAD VIRUS DETECTION: STRIP TEST

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Simple immunobased test kit for yellow head virus (YHV) detection was developed using (1) V3-2B monoclonal antibody conjugated to colloidal gold laid on sample pad (2) rabbit anti-YHV antibody (T) as a capture antibody laid on nitrocellulose strip (3) goat anti-mouse IgG antibody (GAM) as control line laid next to the rabbit anti-YHV antibody (C). The test can be easily performed by adding sample (haemolymph or tissue extract mixed with prepared anti-coagulant buffer) into the well mixed with colloidal gold conjugated antibody on sample pad and let the solution flow pass the capture antibody. If YHV is present in the solution, it will bind to conjugated antibody and the Ag-Ab complex will be caught by the capture antibody and appears as red purple line on the T region. The excess unbound conjugated antibody will flow pass this line and will be captured by GAM at C region. The result can be observed within a minute. This prototype of the kit is suitable monitoring YHV infection in the farm. Further improvement for more sensitivity kit is undergoing development.

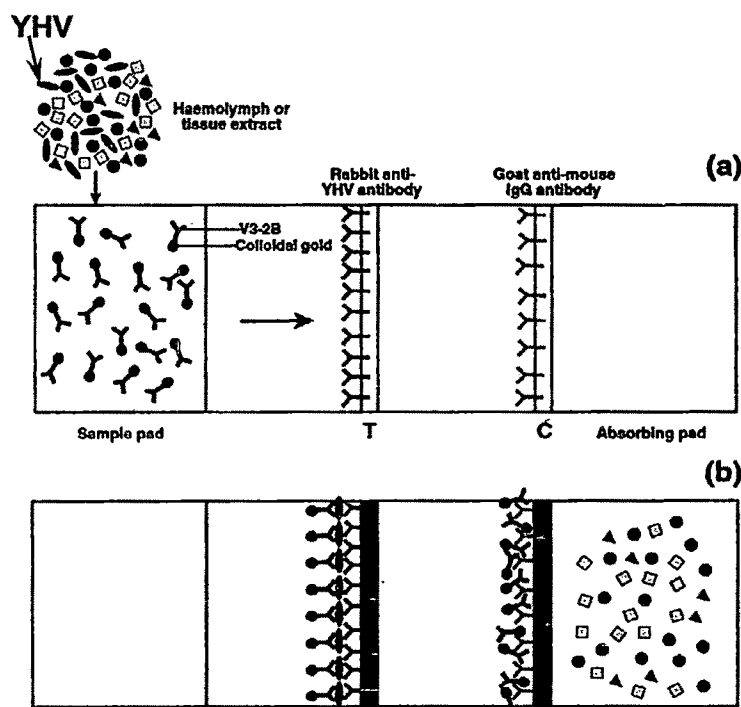


Figure 1 Diagram showed (a) position of various antibodies in strip test kit. (b) After application of a sample containing YHV, conjugated antibody and antigen complex was captured by the rabbit anti YHV antibody on the test line (T) and the excess conjugated antibody was captured by goat anti mouse IgG antibody on control line (C) and the rest of solution accumulated on absorbing pad.

GENERATION OF MONOCLONAL ANTIBODIES SPECIFIC TO HEPATOPANCREATIC PARVOVIRUS (HPV) FROM *Penaeus monodon*

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Hepatopancreatic parvovirus (HPV) was isolated from the hepatopancreas of slow growth *Penaeus monodon* by urografin gradient centrifugation. The presence of HPV in the fraction was monitored by PCR and SDS-PAGE. Only one major 54 kD protein band was observed in the strong PCR-positive fractions used to immunize in mice for monoclonal antibody production. After cell fusion, the first step selection for specific antibodies was performed by dot-blot assay with purified HPV viral particles. The second screening step was done using Western blots of purified HPV proteins and immunohistochemistry of HPV-infected HP tissue. Four monoclonal antibodies were isolated. These bound to the 54 kD protein in Western blots and to intranuclear inclusion bodies in tubule epithelial cells of HPV-infected prawn tissue by immunohistochemistry. None of the antibodies showed cross-reactivity to either uninfected shrimp tissue or to other shrimp viruses tested. These reagents have potential for use in developing a highly sensitive immunoassay such as sandwich ELISA or a convenient kit for detection of HPV infection.

Table 1 Characterization of monoclonal antibodies specific to HPV

MAb (subclass)	Immuno detection method			Cross-reactivity to other viruses			
	Dot blot	Western blot	IHC	YHV	YHV (nonvirulent)	WSSV	MBV
HPV6-12B (IgG _{2b})	+	++	++	-	-	-	-
HPV12-10C (IgG ₁)	+	+	+	-	-	-	-
HPV16-9C* (IgG _{2b})	+	++	++	-	-	-	-
HPV 17-1G (IgG ₁)	+	+	+	-	-	-	-

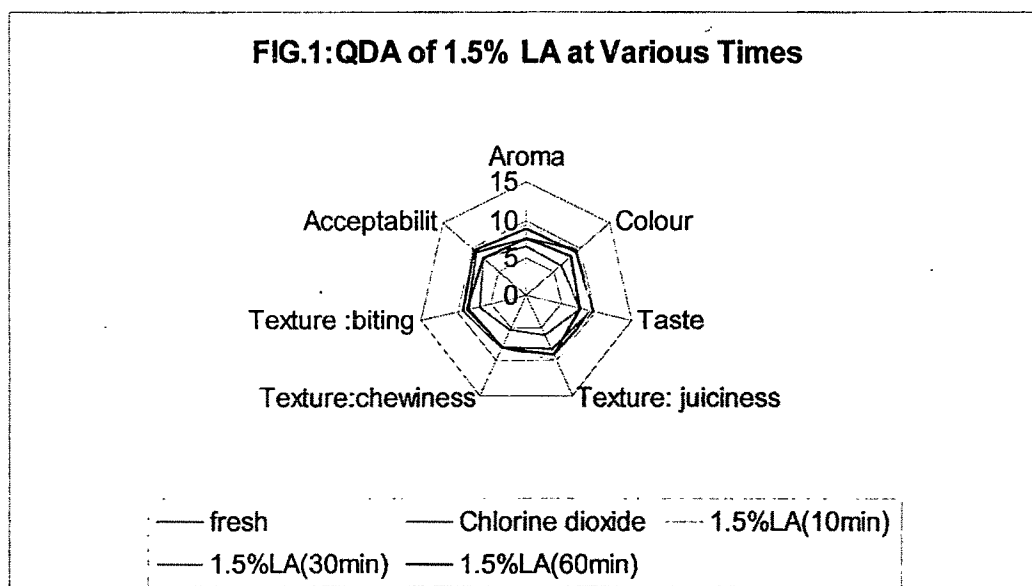
*represent antibody used for characterization; - = no immunoreactivity;
+ = light immunoreactivity; ++ = strong immunoreactivity; IHC = Immunohistochemistry

THE USE OF L - (+) - LACTIC ACID AND SODIUM - L - LACTATE FOR THE CONTROL OF MICROBIAL QUALITY IN PRAWNS *Penaeus merguensis*

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Lactic acid (LA) and its derivatives (mainly sodium lactate (NaL)) are generally regarded as safe (GRAS) and are known to have antimicrobial activity towards gram-negative bacteria such as *Salmonella enteritidis*, *E.coli* spp. and *Vibrio* spp., the common causes of food contamination. In recent years lactic acid and sodium lactate have been regularly used in the meat processing industries as antimicrobial agents, particularly during handling and storage. Although known microbial hazards exist in the seafood industry, particularly as *Vibrio* species, only a few studies have reported use of lactic acid for their control. Hence this study was carried out to examine the effectiveness of L (+)-LACTIC ACID (PURAC®) and SODIUM - L - LACTATE (PURASAL® S) as antimicrobial agents towards *Salmonella enteritidis*, *E.coli* strains and *Vibrio* species. Fresh and chilled prawns previously inoculated with a predetermined microbe (log 7-9 cfu/g) were dipped in solutions of 0, 1.5 and 3.0% lactic acid and 2.5 % buffered lactic acid. There was a reduction in colony counts as far down as log 3 cfu/g, depending on the contact time from 1 to 30 min. Frozen prawns inoculated with an initial load of each selected microbe, were glazed with solutions containing 0, 0.5 and 2.5% sodium lactate and 2.5% buffered lactic acid and kept frozen at -20°C for 30 days. Studies showed that 2.5% buffered lactic acid with a pH value of 4.51 was able to reduce *E.coli* by 14.9%, *Salmonella* by 21.6%, *V. paraheamolyticus* by 9.8% and *V. cholerae* by 12.7%. On the other hand, sodium lactate with a pH ranging from 6.62 to 8.4 was not effective as an antimicrobial. Contrary to an earlier belief that sodium lactate causes moisture loss; buffered lactic acid was able to reduce moisture loss to less than 1%. The effects of using lactic acid and sodium lactate on sensory qualities of fresh and frozen *Penaeus merguensis* showed that fresh prawns dipped in 1.5% LA for 10 min were highly acceptable while frozen samples glazed in 2.5% buffered LA were most acceptable to trained panelists (Fig. 1).

FIG.1: QDA of 1.5% LA at Various Times



DETECTION OF PM-SYNTENIN PROTEIN IN *Penaeus monodon* BY SPECIFIC ANTIBODY

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Full length cDNA of Pm-Syntenin was isolated from hemolymph of black tiger shrimp (*Penaeus monodon*) infected with white spot syndrome virus (WSSV) and sub cloned into *Escherichia coli* M15. Recombinant protein purified from *E. coli* M15 had a molecular mass of approximately 35 kD and was used to immunize rabbits to obtain a specific antibody. The expression of Pm-Syntenin in hemolymph and various tissues of normal and WSSV-infected shrimp were subsequently followed. The results showed that Pm-Syntenin expression levels were not different in tissues of normal and WSSV-infected shrimp. The antibody reacted to two protein bands (M_r 70-72 and 35 kDa) in tissues but to a single protein band (M_r 70-72 kDa) in hemolymph. The molecular weight of 70-72 kDa protein was higher than that predicted by the coding region of Pm-Syntenin.

THE LYMPHOID ORGAN OF GROWTH-RETARDED *Penaeus monodon* CONTAINS YELLOW-HEAD-LIKE VIRUS

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A new and unusual type of growth retardation has been reported in cultured black tiger shrimp (*Penaeus monodon*) in Thailand and has caused serious loss to the shrimp culture industry since 2002. A case definition stipulates that a suspected population must have a coefficient of variation (CV = standard deviation/mean) of more than 35% by weight and absence of hepatopancreatic parvovirus (HPV) infection or of other severe HP infections while also complying to any 3 out of the 5 following gross signs: 1) unusually dark color, 2) average daily weight gain of less than 0.1 g/day at 4 months, 3) unusually bright yellow markings on body, pleopods and antennae, 4) bamboo segments, 5) brittle antennae. The problem has been found to be unassociated with known pathogens so other potential causes are being investigated. Lymphoid organs (LO) of growth-retarded *P. monodon* were aseptically removed and homogenized in buffer and the supernatant LO extract (LOE) solution was sterilized by filtration through a 0.45 μ m membrane. LOE or carrier buffer (controls) was injected intramuscularly (0.05 ml) into 1-2 g domesticated *P. monodon* that were free from 5 shrimp pathogenic viruses by PCR and RT-PCR assay. A yellow-head virus (YHV) stock solution was injected into one group of shrimp as a positive control. At 90 days, mean body weight, survival rate and coefficient of variation (CV) for mean body weight were determined. The lymphoid organs were prepared and observed by light microscopy (LM) and transmission electron microscopy (TEM). LOE-injected shrimp showed gross signs similar to those described above for growth-retarded shrimp in the field and mean body weight ((BW = 8.1 ± 0.7) and survival rate (SR = $53.3 \pm 11.1\%$) was significantly lower ($p < 0.05$) than in the control group (9.8 ± 0.2 g and $86.7 \pm 8.4\%$, respectively) while CV for mean body weight was significantly higher ($38.8 \pm 5.4\%$) than in the controls ($21.7 \pm 3.9\%$). LM examination of LO revealed abnormal cells with fragmented nuclei, cellular vacuolization and small to very large, magenta, cytoplasmic inclusions in spheroids. TEM of LO revealed numerous yellow-head-like viral capsids but no mature virions in the cytoplasm. By contrast, shrimp injected with YHV stock showed nucleocapsids together with mature virions. Other spheroidal particles ranging from approximately 14 to 45 nm in diameter were also seen in the TEM sections from both LOE-injected and control shrimp. The role of these particles and yellow-head-like virus particles in unusual growth-retardation in *P. monodon* is still inconclusive.

**DETECTION OF SUPEROXIDE AND NITRIC OXIDE IN KURUMA SHRIMP
Marsupenaeus japonicus HEMOCYTES**

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To detect superoxide in shrimp hemocytes, a *Cypridina* luciferin analog (CLA) that reacts specifically with singlet oxygen and superoxide was highly sensitive as a chemiluminescence probe but luminol was not sensitive when measured by a chemiluminescence detector. The use of EDTA and citrate as an anticoagulant for shrimp hemolymph did not affect the CLA-dependent chemiluminescence (CDC) but cysteine inhibited it. CDC of shrimp hemocytes was stimulated by phorbol myristate acetate. Shrimp hemocyte CDC was inhibited by superoxide dismutase and EDTA, but not by DABCO and NaN_3 ; both of which are singlet oxygen quenchers. CDC of granulocytes, which are the most active phagocytes among three different types of hemocytes in shrimp, was much higher than that of other hemocyte types. Shrimp infected with white spot syndrome virus showed a decrease in CDC. From these results, we concluded that shrimp granulocytes can produce superoxide so that pathogens ingested by them may be killed by super oxide. Nitric oxide (NO) production from shrimp hemocytes was detected by fluorometric measurement after co-stimulation with lipopolysaccharides (LPS) and recombinant human interferon γ *in vitro*. The supernatant of a hemocyte culture after stimulation showed an increase in fluorescence intensity, and this intensity decreased with the addition of N^G -monomethyl-L-arginine (L-NMMA). These results show that shrimp hemocytes can produce O_2^- and NO and that these free radicals play an important role in the shrimp defense network.

SELECTION OF YHV-BINDING PEPTIDES BY PHAGE- DISPLAY AND TESTING FOR PROTECTION AGAINST YELLOW HEAD VIRUS INFECTION IN BLACK TIGER SHRIMP

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Yellow head virus (YHV) causes a serious disease that can result in high mortality of penaeid shrimp within 2-3 days after the first appearance of gross signs of disease in rearing ponds. Current detection systems for YHV are based on molecular and immunological techniques. The former required sophisticated equipment and well trained lab personnel while the latter can be adapted for simple pond-side assays appropriate for use by shrimp farmers. However, current immunological detection methods require continual production of relatively large, complex proteins using living animals or hybridoma cells. An alternative possibility is to use the phage display technique to find short peptides that could bind strongly with yellow head virus and replace antibodies in such tests. Once identified, these peptides could also be tested for efficacy in inhibiting YHV infection, much in the same manner as reported for egg yolk immunoglobulin (IgY). YHV purified at 4°C was immobilized on plates and exposed to a pool of phage-generated peptide variants. Unbound phage variants were washed away while specifically bound variants were eluted. Cyclic repetition of this process with sequentially generated phage pools resulted in selection of variants with the strongest binding sequences. Subsequent isolation of individual clones will be followed by DNA sequencing and characterization of the bound peptides. Their target viral proteins will then be identified by Western blot analysis for selection of candidate peptides to be used in YHV detection and infection inhibition tests.

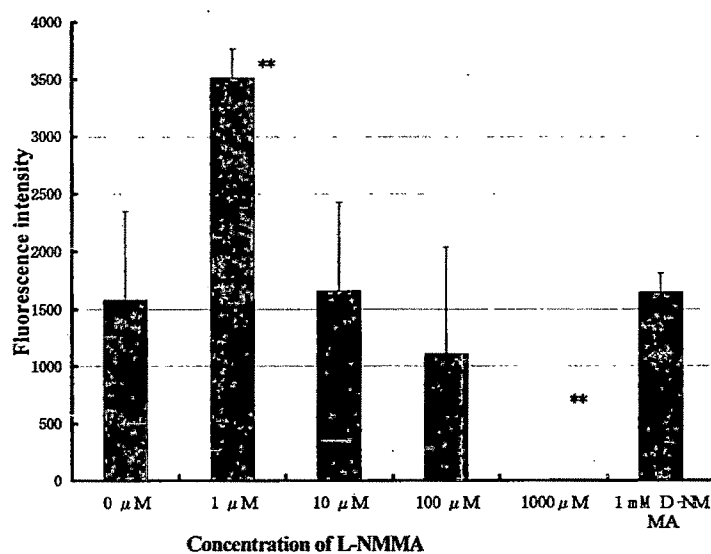


Figure 1 Inhibitory effect of L-NMMA on nitric oxide production from shrimp hemocytes co-stimulated with LPSe and rhIFN γ . Bars indicate average fluorescence intensity ($\pm$ SD, n=3) of three shrimp. Asterisks represent statistically significant differences in fluorescence intensity between groups with L-NMMA added or not added. **: $p < 0.01$

STUDY OF MONODON SLOW GROWTH AGENT (MSGA) A PROBABLE NEW SHRIMP PATHOGEN IN THAILAND

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In 2002, Thai shrimp production fell about 40 percent (160,000 tons) from 2001 mostly due to unusual, widespread and unexplained slow growth in the black tiger shrimp (*Penaeus monodon*) called monodon slow growth syndrome (MSGS). The phenomenon appeared quickly and in a wide variety of cultivation systems, suggesting that a new or existing infectious agent might be the cause. A large scale screening program revealed several known viral pathogens (MBV, HPV and IHNV) and one previously un-described microsporidian in MSGS ponds, but none were significantly correlated with MSGS. Lymphoid organ (LO) spheroids of some of the MSGS shrimp showed unusual, large, magenta cytoplasmic inclusions. Transmission electron microscopy (TEM) of lymphoid organ (LO) tissues of slow growing shrimp revealed the presence of 5 types of potential viral particles. The first type was filamentous cytoplasmic particles resembling early replication stages of yellow head virus (YHV) while the other 4 were spherical cytoplasmic particles of approximately 14 nm, 22 nm, 30 nm and 45 nm diameter. Particles of 14 nm and 30 nm were not seen in a previous TEM study of *P. monodon* LO preparations in Thailand. The 30 nm particles somewhat resembled viral-like particles described by D.V. Lightner in late 2002 from the LO of captured *P. monodon* broodstock from the Andaman Sea. RT-PCR tests for YHV revealed a unique variant not previously reported from Thailand, but a subsequent field survey with a specific RT-PCR assay for this variant revealed no significant correlation with MSGS. At the same time, bacteria-free LO extracts from slow growing shrimp that were injected into domesticated *P. monodon* caused severe growth retardation and extreme size variation in the first two months of cultivation. The experiments suggested that a filterable agent called MSGA was the cause. Other unusual gross signs in the injected shrimp led to the following case definition for MSGS: the suspected population must have a coefficient of variation (CV = standard deviation/mean) of more than 35% by weight and absence of hepatopancreatic parvovirus (HPV) infection or of other severe HP infections while also complying to any 3 out of the 5 following gross 1) unusually dark color, 2) average daily weight gain of less than 0.1 g/day at 4 months, 3) unusually bright yellow markings, 4) bamboo segments and 5) brittle antennae. Putative viral particles were separated from LO extracts of MSGS shrimp and subjected to gradient centrifugation and putative viral bands were subjected to total nucleic acid extraction for randomly primed RT-PCR amplification. Amplicons are being cloned and screened by hybridization with labeled shrimp DNA and those that do not hybridize are being sequenced for a GenBank search of pathogens previously unreported for *P. monodon*.

**GENOMIC ANALYSIS OF A NEW MICROSPORIDIAN THAT INFECTS THE BLACK
TIGER SHRIMP *Penaeus monodon***

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The Microsporidia are obligate intracellular, spore-forming parasites infecting many types of animals including insects, birds, fish, crustaceans and mammals. The black tiger shrimp *Penaeus monodon* is rarely infected by microsporidia. However, during 2002-2004, several Thai *P. monodon* farms reported infections by multiple pathogens, including viruses, bacteria and a new hepatopancreatic microsporidian. Infected hepatopancreatic cells contained basophilic microsporidian spores in the cytoplasm, some within membrane-bound sporophorous vesicles. By transmission electron microscopy (TEM), spores were oval to pyriform or reniform with average dimensions of 0.8x0.6 μ m. The spore wall was three-layered and a single nucleus, sporoplasm, anchoring disc and polar tube were seen. The polar tube was coiled 3-5 times around the nucleus and the anchoring disc was composed of alternate electron-dense (2 nm) and electron-lucent (30 nm) layers. Negative staining revealed a protruding polar tube. These ultrastructural features resembled those of the microsporidian species *Perezia nelsoni* and *Tuzetia weinaei*. Microsporidian spores were freshly isolated from the hepatopancreas by homogenization in sterile water (1 g: 100 ml) and centrifugation at 590 x g and 4°C for 20 min. The pellet was removed and vortexed with TN buffer before over layering on a discontinuous sucrose gradient and ultracentrifugation at 100,000 x g for 2 hr. The microsporidian spore band was removed and resuspended in TN buffer. Specific primers designed from the rRNA gene of *Agmasoma penaei* (another microsporidian that infects *Penaeus monodon*) were used to amplify genomic DNA isolated from these microsporidian spores. The PCR product was sequenced and shown to be more related to records for fungi (Ascomycetes) than microsporidians.

**SEROTONIN IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM AND
OVARIES OF THE BLACK TIGER SHRIMP *Penaeus monodon***

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Serotonin (5-hydroxytryptamine, 5HT) is involved in modulation of behavior and physiological functions of both vertebrates and invertebrates. It has been reported that 5HT can induce ovarian maturation and spawning in certain shrimp species, but such studies have not yet extended to the black tiger shrimp *Penaeus monodon*. To explore the role of 5HT in *P. monodon*, we used immunohistochemical methods to localize 5HT-immunoreactive cells in the central nervous system and in the ovaries of *P. monodon* broodstock. It was found that 5HT-positive reactions occurred in cell bodies of the brain and thoracic ganglia, as well as in the ventral nerve cord and nerve fibers surrounding the ganglia. A positive reaction was also found in the ovaries within follicular cells at the pre-vitellogenic stage (stage I, II), but not in oogonia or on the cell membrane of mature oocytes (stage IV). It is therefore possible that 5HT released from the thoracic ganglia might act directly on the ovaries of *P. monodon*.

DEVELOPMENT OF POLYCLONAL ANTIBODIES TO POLYHEDRIN OF *Monodon baculovirus* (MBV) USING A SYNTHETIC POLYPEPTIDE

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Monodon baculovirus is a typical nuclear polyhedrosis virus (NPV) that produces polygonal polyhedrin protein particles in the nuclei of hepatopancreatic tubule epithelial cells in the black tiger shrimp *Penaeus monodon*. These protein particles contain embedded MBV virions and are believed to aid in virion protection and dispersal. Polyhedrin protein of *Monodon baculovirus* (MBV) was isolated from MBV-infected post larvae (PL) of *Penaeus monodon* a combination of homogenization by hydroforce and by differential centrifugation. After ultracentrifugation, a band at 50% Urografin was examined by negative staining transmission electron microscopy and shown to contain particles of 22-23 nm diameters that resembled polyhedrin protein subunits previously described. Since these particles also resembled parvovirus virions, the fraction was extracted for DNA and confirmed to be negative for hepatopancreatic parvovirus (HPV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) by polymerase chain reaction assay. Further analysis of this fraction by SDS-PAGE yielded a single major protein band at 58 kDa, corresponding to the molecular weight of MBV polyhedrin as previously reported. The first 25 amino-acid residues at the N-terminus of the isolated protein band were sequenced and a peptide (25PmPV) was synthesized using that sequence. The synthetic peptide was conjugated to bovine serum albumin and used as antigen for antiserum production in mice. By means of immunohistochemistry, the mouse anti-25PmPV antiserum showed strong immunoreactivity to occlusion bodies of MBV in tissue sections of infected PL but not to inclusions of HPV, another common viral pathogen of the hepatopancreas. Nor did it react with intranuclear inclusions of white spot syndrome virus in gill tissue sections. These evidences confirm that the isolated protein and sequence of the peptide are from polyhedrin protein of MBV. These results lay the foundation for further work in preparing monoclonal antibodies specific to MBV polyhedrin.

OUTBREAKS OF TAURA SYNDROME VIRUS (TSV) WITH EXOTIC *Penaeus vannamei* CULTIVATED IN THAILAND

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The cultivation of exotic *Penaeus vannamei* in Thailand began on a very limited scale in the late 1990's, but a Thai government ban on the cultivation of *Penaeus monodon* in fresh water areas in 2000 led many shrimp farmers there to shift to cultivation of *P. vannamei*. High growth rates and high yields from imported specific pathogen free (SPF) stocks and dissatisfaction with widespread slow growth in cultivated *P. monodon* in 2001 and 2002 led to even wider adoption of *P. vannamei* as an alternative. Alarmed by the possibility of TSV introduction, the Thai Department of Fisheries required that imported stocks of *P. vannamei* be certified free of TSV by RT-PCR testing. During the interval, over 150,000 broodstock shrimp were imported, 67% from China and Taiwan. In spite of the safeguards, TSV outbreaks occurred and were confirmed by RT-PCR in early 2003. This resulted in a governmental ban on all shrimp broodstock imports from February 2003, but TSV outbreaks have continued, possibly due to the illegal importation of stocks. Examination of the viral coat protein gene VP1 amplified by PCR from several isolates of TSV associated with 10 shrimp disease outbreaks in Thailand revealed that the Thai isolates form a clade distinct from TSV isolates from the Americas. An additional 6 TSV isolates from China formed a clade intermediate between the American and Thai clades, suggesting that TSV arrived in Thailand via China or Taiwan rather than directly from the Americas.

**IMMUNOLOCALIZATION OF THE PROSTAGLANDIN F2 ALPHA RECEPTOR IN THE
OVARY OF *Penaeus monodon* BROODSTOCK**

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Prostaglandin F2 alpha (PGF2 α) is considered an important mediator of reproductive physiological processes in several invertebrates, including in the black tiger shrimp *Penaeus monodon*. The hormone stimulates ovarian maturation and spawning in *P. monodon* and binds to a specific PGF2 α (FP) receptor. In this study we used a specific antibody against FP receptor to localize the receptors in various developmental stages of the ovary of *P. monodon* broodstock by using the immunoperoxidase technique. The result revealed that FP receptor immunoreactivity was localized on both the cell membrane and the nuclear membrane of oocytes in the pre-vitellogenic stage of ovarian development. In the vitellogenic stage, the activity was localized on the cell membrane and also on cortical rods of oocytes. On-going activities are focused on how PGF2 α influences ovarian maturation and spawning, including molecular events linking the binding of its receptors to the oocyte maturation process.

DOMESTICATION OF SLENDER BETTA *Betta bellica* AND ITS POTENTIAL FOR COMMERCIALISATION

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Betta bellica is one of the black water ornamental fish species in the group of Anabantidae. Studies of *Betta bellica* breeding in captivity and its potential for aquarium industry were initiated in 1994. The fish has a bright future for the aquarium fish industry but is facing extinction owing to the over exploitation of wild stocks and uncontrolled land development for agriculture, especially in peat soil areas. Endemic to swampy peat soil areas, the species thrives in tannin-stained water with pH around 4.5 - 6.5 especially in the southern part of the Malaysian Peninsula. The species is known to be very difficult for captive breeding but is peaceful among members of its own species in a community tank.

The objectives of the current study were to domesticate wild caught *B. bellica* specimens and to formulate a captive breeding method for mass production of fry. Samples of the fish were acquired from fish collectors in Ayer Hitam, Johore. Wild samples were domesticated in glass aquaria for conditioning. During the domestication process, specimens were fed with live feeds such as bloodworm, moina and tubifex worm. The fish attained maturity and were suitable for spawning after 2 months in captivity.

A bubble-nest builder, spawning took place in aquarium filled with water acidified to pH about 5.5 - 6.5 with sphagnum peat moss. The fish spawned underneath the bubble-nest producing cream-coloured fertilised eggs in the nest. Females about 10 cm in length can produce about 200 - 250 eggs. At a temperature about 27°C the eggs hatched between 24 - 30 hours. The yolk sac stage was about 3 - 4 days. The fry were free-swimming by the fourth day. The percentage of hatching ranged from 60 - 70%. First feeding with fine live food, such as *Artemia* or infusoria, was carried out on the fourth day, followed by larger live food such as *Moina* sp for another week. Within 1 month, the fry attained a size of about 1.0 - 1.5 cm. The fry can be marketed after 4 months' culture in 90 x 45 x 45 cm aquarium tank at about 6.0 - 7.0 cm length.

POPULATION GENETIC RESEARCH ON ORNAMENTAL FISH AT UNIVERSITY OF MALAYA

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In 1999, the Institute of Postgraduate Studies and Research (IPSR), University of Malaya started an ornamental fish genetic project involving the Asian arowana (*Scleropages formosus*) angel fish (*Pterophyllum scalare*) and koi (Japanese carp) to identify the genetic characterization of these ornamental fish. In addition, a population genetic programme was carried out to monitor the genetic variability of the ornamental fish. In this report, the focus is given to the genetic variability study of arowana and angel fish which have been completed.

Five strains of arowana, namely green, red-tail Malaysian gold, yellow-tail Malaysian gold Indonesian gold and red arowana were screened using twenty-nine microsatellite loci. On the other hand, four strains of angel fish (silver, light marble, gold marble and koi) were evaluated using twenty-three isozyme loci.

The number of alleles in every strain of arowana was low. Five strains of arowana showed different degree of polymorphism. The red-tail Malaysian gold arowana showed the highest allele number while the green had low number of alleles. There were significant deviations from Hardy-Weinberg equilibrium in every strain of arowana and high level of F_{IS} . The Malaysian yellow-tail gold arowana experienced the highest degree of deviation from Hardy-Weinberg equilibrium while the Indonesian gold arowana showed the lowest degree.

The number of alleles in the angel fish was lower than the arowana. The highest number of alleles was found in the silver strain while the gold marble strain showed the lowest number of alleles. There was significant deviation from Hardy-Weinberg equilibrium in all the strains with highly significant F_{IS} values.

Estimates of genetic variability and coefficient of inbreeding showed that captive breeding programme has caused departure from the Hardy-Weinberg equilibrium. Improvement of the genetic management by means of monitoring the genetic variability estimating precise effective population size can be the solution to minimize genetic impacts of inbreeding to these ornamental fish.

THE AGGRESSIVENESS STUDY OF FIGHTING FISH *BETTA UNIMACULATA* AND *BETTA PUGNAX*

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The behavior of the fighting fish *Betta unimaculata* from Balung River in Sabah and *Betta pugnax* from Titi Teras River in Penang are being studied. *B.unimaculata* can only be found on Borneo Island, while *B.pugnax* is only found in Peninsula Malaysia. Comparative studies on the aggressive behavior have been conducted between the male and female of *B.unimaculata* and *B.pugnax*. *B.unimaculata* and *B.pugnax* were obtained from the river by using scoop net and packed in plastic bags. In the laboratory, both species were kept in different aquarium. In the first experiment, each species was given a mirror and their response towards their own mirror image was recorded. In the second experiment, two different species or sexes were put in the same aquarium, which was separated by a transparent film. The duration of each of the fish showing aggressiveness was recorded. The experiments were conducted before and after feeding.

It is found that the *B.unimaculata* was more aggressive than *B.pugnax*. In addition, *B.unimaculata* males are more aggressive than the females. Similar response was shown by the male *B.pugnax*, which was more aggressive than the female *B.pugnax*. Males of *B.unimaculata* and *B.pugnax* took shorter time to attack food compared to the females of both species. *B.unimaculata* males took longer time to complete feeding compared to the females. While, *B.pugnax* males took shorter time to complete feeding compared to the females.

Table 1 Comparison between male *Betta unimaculata* and female *Betta unimaculata* with male *Betta pugnax* and female *Betta pugnax*.

Parameter	Time			
	<i>Betta unimaculata</i>		<i>Betta pugnax</i>	
	Male	Female	Male	Female
Show aggressiveness before feeding	13.3s ± 4.2s	44.3s ± 9.9s	12.7s ± 10.0s	12.0s ± 7.3s
Show aggressiveness after feeding	1min 8.0s ± 13.5s	30.0s ± 8.0s	14.5s ± 6.5s	22.5s ± 9.5s
Attack food	1.5s ± 0.5s	3.5s ± 1.5s	1.5s ± 0.5s	3.5s ± 1.5s
Stop eating	8min 50.0s ± 1min 8.0s	4min 32.0s ± 37.0s	2min 8.0s ± 27.0s	4min 21s ± 41.0s

CAPTIVE BREEDING PERFORMANCE OF CLOWNFISH *Amphiprion ocellaris* IN MALAYSIA

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Production of organisms for the aquarium hobbyist trade is a rapidly growing sector of the aquaculture industry and it will become more important as restrictions are placed on collecting wild population with direct negative repercussions especially on coral reefs and wild ecosystems. Clownfish, *Amphiprion ocellaris* is considered one of the most popular marine ornamental fish in the world. Most of the markets production is wild caught population.

The present study illustrates the first successfully study on breeding and rearing of a small Pomacentridae, *Amphiprion ocellaris* in Malaysia in captivity. Broodstock were collected at selected sites of Bidong Island, Terengganu. Fish were kept in 3.0×1.5×1.0 m concrete tank connected to enclosed water recirculation system for 4 to 8 weeks to acclimated captive condition. After acclimated period, 15 pairs mature broodstock were selected based on morphological maturation criteria at ratio 1:1 and distributed randomly into 2 feet breeding tanks equipped with flow-through recirculation filter system. Water quality in breeding tanks were maintained at temperature ranged from 26°C – 28°C, salinity at 28 – 30 ppt, pH at 8.0 – 8.2 and dissolved oxygen between 6.0 – 8.0 mg/L, respectively. Two substrates were provided into each spawning tank where included natural coral rock and PVC pipe cut into 15 cm.

Spawning pairs were selected after 4-8 weeks acclimation in hatchery condition. A spawning pair will clean out a selected site and cleaning process will take 1 to 2 hours before actual spawning. Total number of 15 pairs broodstock were selected for breeding study, 11 pair were successful form, 8 pairs were successful spawning with 53.33%, while 3 pairs were eggs eaten consisted of 20%. The less were un-spawning consisting of 4 pairs in 26.67%.

Spawning usually take place during the afternoon from 1400 to 1900 hours with about 100-800 numbers of eggs for each pair. The eggs are demersal and elongate ovoidal in shape, incubation period take time about 6-7 days and hatching took over a total time of 12 - 24 hours. Newly hatched larvae were able to swim and positive phototactic in size of 3.8 – 4.2 mm with mouth size 0.4 mm, which is able to fed on copepods and *Artemia* nauplii. Average hatching rate were 95.97%, survival rate were recorded at 67.67%, respectively. Newly hatched larvae should be fed immediately with rotifer on day 1 and 2.

OUTBREAK OF KOI HERPESVIRUS INFECTION IN JAPAN

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Mortality among common carp (*Cyprinus carpio*) for food reared in net pens in Kasumigaura, the second largest lake in Japan, in Ibaraki Prefecture, has rapidly increased since early October 2003. An examination of moribund fish in late October by the National Research Institute of Aquaculture (NRIA) demonstrated the presence of KHV by PCR methods of Gilad *et al.* (2002) and Gray *et al.* (2002). The apparent changes in the affected fish were similar to those reported by Hedrick *et al.* (2000), such as excess mucus secretion on the body surface, sunken eyes, discoloration and clubbing of the gill filaments.

Subsequently, the disease spread rapidly to 23 prefectures by the end of 2003. No outbreak of the disease was observed during the winter period. However, as the water temperature increased in the spring of 2004, KHV infection reappeared in the area where the disease had been recorded previously and in new places, causing massive mortality of both cultured and wild carp. By the end of June 2004, KHV infection was reported in 33 prefectures in Japan. To date the disease has not been observed in farms of ornamental carp (Koi).

KHV has been isolated from the gill or kidney of affected fish in seven independent cases using the KF-1 or CCB cell line. There is no known epidemiological relationship among these cases. These virus isolates were virulent to common carp in experimental infections by intraperitoneal injection and bath exposures. Nucleotide sequences of the three regions (KHV9/5 primer set amplicon; 37 cases, SphI-5 primer set amplicon; 12 cases and thymidine kinase; 24 cases) of KHV DNA detected in Japan were identical, and those in Indonesia and Taiwan were also the same as the isolates from Japan. The KHV isolates from Japan differed from KHV from Israel and the Netherlands in nucleotides in three regions.

16S rRNA SEQUENCE ANALYSIS OF *Edwardsiella tarda* STRAINS

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The primers were designed for rapid identification of *Edwardsiella tarda* by PCR from the analysis of 16S rRNA sequences of various strains. Seven cultured strains of *E. tarda* were used in this study. Six of these strains are pathogenic for the fishes; E22 (eel: *Anguilla japonica*), E381 (tilapia: *Tilapia nilotica*), NB8030 (red sea bream: *Pagrus major*), NE8003 (Japanese flounder: *Paralichthys olivaceus*), TC165 (ayu: *Plecoglossus altivelis altivelis*) and TC222 (gizzard shad: *Clupanodon punctatus*). Another strain of SU138 (eel) is low-pathogenicity against the fish. The DNA was extracted from these strains and 16S rRNA were amplified by PCR using universal primers and sequenced.

Phylogenetic relationship was inferred among determined sequences of seven strains and other *Edwardsiella* species on the database. The 16S rRNA phylogeny showed two clusters. One cluster was formed by SU138 strain of *E. tarda*, *E. hoshinae*, and *E. tarda* strains that are isolated from land animals. Another cluster was composed by *E. ictaluri* and the other *E. tarda* strains determined their 16S rRNA sequences. Members of the former cluster were thought to be of low or non-pathogenicity to the fishes. On the other hand, the latter cluster group consisted of pathogenic strains in *Edwardsiella* species.

The primers were designed for the specific regions in 16S rRNA gene to distinguish between these clusters. The results of PCR using these primers, *E. tarda* was able to be recognized specifically, and also cluster specific amplification was observed on *E. tarda* strains corresponding to their belonging cluster group, respectively. The primers could use for detection of *E. tarda*, and it seemed to be able to infer of its pathogenicity.

ENHANCED ONE-STEP POLYMERASE CHAIN REACTION FOR DETECTION OF KOI HERPESVIRUS

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Koi herpesvirus (KHV) has been causing massive mortalities in koi *Cyprinus carpio koi* and common carp *Cyprinus carpio carpio* in Israel since 1998. The disease was later reported in the USA and Europe. The first suspected outbreak in Asia was reported in Indonesia in 2002. Gilad *et al.* (2002) has reported the detections of KHV by one-step polymerase chain reaction (PCR). The present study attempted to enhance the sensitivity of the one-step PCR assay reported by Gilad *et al.* (2002) by re-optimizing the concentrations of reaction components and thermal cycling profile, using 10-fold serial dilutions of DNA fragment clone of KHV isolate from Indonesia as templates.

The re-optimized assay amplified the 484-bp target amplicon from 10^{-1} to 10^{-7} dilutions (Fig. 2, lanes 3 to 9), indicating 100,000-fold increment of assay sensitivity compared to the original assay that only showed positive reactions in 10^{-1} to 10^{-2} dilutions (Fig. 1, lanes 3 to 4).

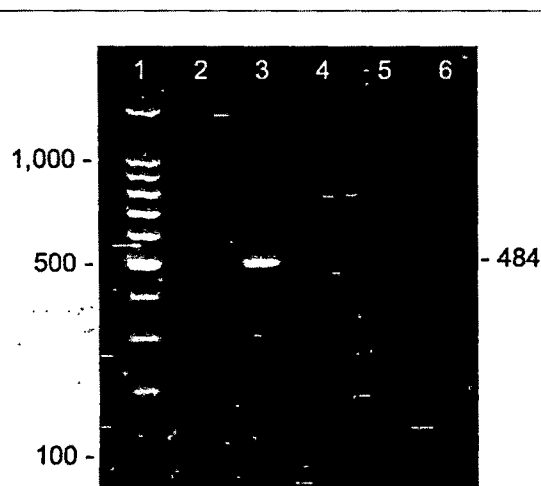


Fig. 1. PCR sensitivity profile before re-optimization. Lane 1: 100-bp DNA ladder. Lane 2: negative control. Lanes 3 to 6: 10^{-1} to 10^{-4} dilutions of DNA templates. Positive reactions were only obtained from 10^{-1} and 10^{-2} dilutions

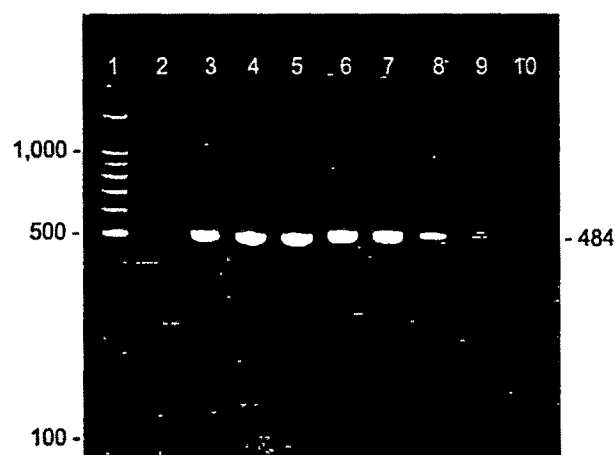


Fig. 2. PCR sensitivity profile after re-optimization. Lane 1: 100-bp DNA ladder. Lane 2: negative control. Lanes 3 to 10: 10^{-1} to 10^{-8} dilutions of DNA templates. Positive reactions were obtained from 10^{-1} to 10^{-7} dilutions, indicating 100,000-fold increment of assay sensitivity compared to the original assay.

BACTERIAL FLORA OF KOI *Cyprinus carpio koi* AND THE ASSOCIATED ANTIBIOGRAM

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Koi *Cyprinus carpio koi* is currently one of the most popular ornamental fish in the world. Koi of many varieties are currently bred in captivity in fish farms in the states of Selangor, Perak, Pahang, Penang and Johore in Malaysia for the export and local markets. Koi farmers and fish retailers commonly apply various types of antimicrobials to koi for prophylaxis for controlling bacterial diseases. The present survey was undertaken to study the bacterial flora of koi and their antibiograms. Bacteria were isolated using selective (TCBS, GSP, DHL, MacConkey, Levine EMB, Baird-Parker and Brolacin) and non-selective (TSA) agars. Identifications were done using conventional methods in combination with BBL Crystal™ Kit. Bacterial isolates were tested using disc diffusion test for susceptibility to novobiocin 5µg, sulfamethoxazole 25µg, ofloxacin 5µg, chloramphenicol 30µg, cefotaxime 3µg, nalidixic acid 30µg, doxycycline 30µg, furazolidone 15µg and amoxycillin 30µg.

The bacterial flora of koi was comprised mainly of *Aeromonas hydrophila*, which is commonly associated with ulcers and hemorrhagic septicemia in koi. Other bacteria isolated were *Vibrio cholerae*, *Enterobacter sakazakii*, *Flavobacterium odoratum*, *Flavimonas oryzihabitans*, *Enterobacter cloacae* and *Escherichia coli*. In general, all isolates were susceptible to the antimicrobials tested except for novobiocin and sulfamethoxazole. This suggested the effectiveness of the antimicrobials for chemotherapy of the associated bacterial diseases.

POTENTIAL FRESH WATER ORNAMENTAL FISHES AND THEIR ABUNDANCE IN THE MUVATTUPUZHA RIVER, KERALA, INDIA

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Fresh water fish fauna of India is diverse and varied. More than 200 species of fresh water fishes having high prospects in the ornamental fish trade have been reported recently from Kerala. The lucrative ornamental fish trade has been utilising numerous wild species in the developing countries. Though resources in India are diverse and rich, contribution to the domestic and international ornamental fish trade is very meager. The trade of ornamental fishes in inland market is met with farm bred exotic species and not with indigenous species. Even if these fishes are not collected from the water bodies, many of them will face natural mortality during the summer season with the drying of rivers and streams. Collection of fish from the wild needs information on its resource status in terms of its abundance and sustainability. Studies on the fish fauna, their abundance and distribution in the different water bodies help to group them based on IUCN categorisation. A sustainable exploitation from the wild is possible only after a thorough knowledge of the biology also.

The present paper is an attempt to identify the fishes in the Muvattupuzha River, in Ernakulam district, Kerala, India, that are suitable for aquarium trade. Observations on fish fauna of the river for a period of two years showed the presence of 61 species, including a new species. There are some key species that can be recommended for ornamental fish marketing. Fishes like *Puntius filamentosus*, *P. mahecola*, *P. vittatus*, *Danio malabaricus*, *Horadandia attukorali*, *Parluciosoma daniconius*, *A.plocheilus blocki*, *A. panchax*, *A. lineatus*, *Etroplus suratensis*, *E. maculatus*, *Macropodus cupanuscdayi* and *Tetraodon travancoricus* were found abundant in the study. They can be caught from the wild for ornamental fish trade, considering their conservational aspects. There were common forms like *P. amphibius*, *Lepidocephalus thermalis*, *Horabagrus brachysoma*, *Anabas testudineus*, *Channa orientalis*, *C. striatus* and *Glossogobius giuris*. Most of these species can be judiciously harvested. Artificial culture and breeding techniques need to be developed for the rare forms like *P. fasciatus*, *Gonoproktopterus curmuca*, *Barilius bakeri*, *Chela dadiburjori*, *Chela fasciata* and *Parambassis dayi*, that have a high demand in the ornamental fish trade. The very rare forms like *Esomus malabaricus*, *Wallago attu* and *Hetropneustus fossilis* may be given protection in the wild. The present study helps to promote ornamental fish trade and its scope in generating income and employment opportunities.

CONSUMER PREFERENCE AND ATTITUDE TO THE ORNAMENTAL FISH MARKETING IN KERALA, INDIA

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The state of Kerala in India offers wide scope for ornamental fish marketing. The ornamental fish industry of Kerala is segmented into the domestic and export market. The domestic industry depends mainly on exotic fishes- Goldfish, Guppy, Molly, Platy, Sword tail, Oscar, Tetra, Koi carps and so on as people in the state prefer exotic fresh water fishes rather than the indigenous freshwater or marine fishes for their home aquaria.

Experimental market approach assumes that the products i.e., here ornamental fish is a composite of attributes at different levels. Hence, rather than a general demand study, the survey aimed at an understanding of consumer attitude and preferences which will enable innovative ventures into this potential market and enhance the industry in a great way. Consumer survey using pre-tested questionnaire was carried out to find the benefits the respondents perceived to obtain from aquarium fish keeping, type of fishes kept in home aquaria, source which ignited the idea of a home aquaria, their most preferred ornamental fishes (from among the common 15 fishes displayed in the retail shops), the frequency of aquarium shop visit, product attributes they considered to be important from the different levels of the attributes like colour, size, price, variety, compatibility, ease of maintenance and other influences. Product attributes preferred by respondents were found using Likert Scales and statistically analysed. Preferences are also analysed with reference to the demographic determinants - age, sex, income, educational level, family size and pattern and experience in the hobby. Ninety nine per cent of the respondents preferred keeping freshwater aquaria rather than marine. Ranking scales for fishes preferred and further conjoint analysis indicated the increasing demand for exotic fishes like Arowana, Flower horn, Discus, Red parrot among the upper elite class of the domestic industry though Gold fishes, Gourami, Angel, Guppy, Molly are all class favourites. Big sized fishes are now considered to be elegant in aquaria. People preferred purchasing entirely different variety fishes during each purchase and that calls for penetration of new exotic fishes into the market. The study reveals that unlike earlier days now the benefit perceived from keeping aquarium is beautification of house interior with a lively decorative object and as a status symbol rather than for mere hobby purpose. Colour and compatibility are the most preferred attributes but they varied with the demographic determinants. Nuclear family set up and increase in disposable income is attributed to be reasons for the increase in the hobby. The innovative strategies of conducting aquarium shows since 1998 by the Government (the official Government statistics report 163 aquarium shops in various districts of Kerala which has increased to 250 after the aquarium shows), marketing strategies of the entrepreneurs in marketing some fishes as Fenghshui fishes (Arowana, 6 Gold fish & 1 Black moor combination) that bring in prosperity, promotion of indigenous fish *Puntius denisonii* as Miss Kerala and added facilities of service marketing offered have triggered the aquarium keeping trend in the state.

CULTURE OF *Spirulina platensis* FOR TOTAL CAROTENOIDS PRODUCTION BY USE OF UREA AS NITROGEN SOURCE

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Spirulina is one type of alga which contains high levels of carotenoids, especially β -carotene and zeaxanthin; which are particularly important for aquatic animal health, pigmentation and stress resistance. However, the mass production of *Spirulina* is limited by the conventional batch-cultivation system and total carotenoids content is relatively low when nitrate is used as nitrogen source. Hence, these studies were undertaken to determine the efficiency of urea nitrogen source on total carotenoids content, and use of fed-batch system for improved cell mass production.

In the first trial, 5 concentrations of urea (0.125 – 2.0 g/l) were used as nitrogen source for batch-cultivation of *Spirulina platensis* in 15 l cylindrical glass aquaria under natural light conditions for 11-days. The maximum cell mass was found in 0.125-0.25 g urea/l and the lowest growth of the algae was found in 2.0 g urea /l. In contrast, the highest total carotenoids content was observed in the harvested cells from 2.0 g urea /l.

In the second trial, fed-batch cultivation system was used to increase the mass production of *S. platensis* with the medium containing 2.0 g urea/l. Four continuous medium-feed rates (5-20 ml/hr) were studied for growth and total carotenoids production of *Spirulina*. Dried cell mass of algae was increased to 1020.30 mg/l with the feed rate of 5 ml/hr, and the lowest cell mass productions were observed in the systems with the feed rates of 15-20 ml/hr. Total carotenoids content was highest in the feed system of 5 ml/hr within 11 days. From the results of these studies, it was found that cell mass production and total carotenoids content in algal cells was 3.96 and 3.09 times higher than the batch culture system. It was concluded that the fed-batch culture with low-cost nitrogen source is practical for industrial scale *Spirulina* production.

**EFFECT OF VARYING DIETARY PROTEIN CONTENT OF BROODSTOCK DIETS ON
REPRODUCTIVE PERFORMANCE AND EGG CHEMICAL COMPOSITION OF
FLOWERHORN *Cichlasoma* sp.**

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The Malaysian Rajah Cichlid is locally known as the Flowerhorn fish. The Flowerhorn is an incredibly beautiful ornamental fish, with a pronounced hump on its forehead and striking floral-body patterns. This beautiful hybrid is thought to be the end product of cross breeding between various species of *Cichlasoma* sp.. Hence, the Flowerhorn lacks a classified scientific name. There is little known about the nutritional requirements of Flowerhorn. The current study was designed to investigate the effects of dietary protein level on various reproductive aspects of Flowerhorn utilizing semi-purified diets.

Reproductive performance of the Malaysian Rajah Cichlid bloodstocks reared on 6 isoenergetic, semi-purified diets of 30, 35, 40, 45, 50 or 55 % crude protein (CP) respectively, for 3 months in relation to spawning periodicity and fecundity was investigated. Egg protein and lipid concentrations were also determined to identify measures of egg quality. Mean number of days elapsed per spawn in females fed 55% CP diets was 16 days compared to 39 days in females fed 30% CP diets. Fecundity increased significantly ($P < 0.05$) with increasing dietary CP content. The lowest fecundity was 1721 eggs clutch⁻¹ in fish fed 30% CP and the highest fecundity was 6223 eggs clutch⁻¹ in fish fed 55% CP. Regression analysis revealed strong and significant relationships between mean fecundity and final fish weight, however, relationships between varying dietary CP content and various indices of egg size (mean egg diameter and mean egg volume) were weaker.

Egg protein concentration increased with increasing dietary CP content. Mean egg protein concentration in fish fed 30% CP (0.46 ± 0.05 mg egg⁻¹) was significantly lower ($P < 0.05$) than fish fed 55% CP (0.80 ± 0.07 mg egg⁻¹). Lipid and moisture content ranged 0.50-0.68 mg egg⁻¹ and 56.3-69.2 % respectively, and there were no significant difference ($P > 0.05$) among the different dietary regime. Fish fed diets with the highest CP concentration had the highest percentage of total polyunsaturated fatty acids (PUFA). The percentage of eicosapentaenoic acid (20:5n-3) was significantly higher ($P < 0.05$) in fish fed 55% CP, whereas the 30% dietary treatment displayed significantly higher ($P < 0.05$) percentage of palmitic acid (16:0) and palmitoleic acid (16:1n7).

**SEASONAL VARIATION IN GROWTH OF *Gracilaria edulis* IN EARTHEN PONDS,
CHONBURI PROVINCE, EASTERN THAILAND**

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Field observations on the growth rate of *Gracilaria edulis* (Gmelin) Silva were conducted from August 2003 to August 2004, at Ang-Sila district of Chonburi Province. Growth of *G. edulis* was set up at seven stations. Thalli were grown in a plastic cage of 30×40 cm², in three replicates. Higher growth rates were observed in the rainy season to cool-dry season (August 2003-February 2004). Maximum-growth rate obtained was 9.3 ± 0.7 %day⁻¹ in August 2003. Plant growth declined during hot-dry season (March-May 2004) and decreased to a minimum value of -8.2 ± 3.5 %day⁻¹ in April 2004, with temperature of 30-33 °C and salinity of 25-34 psu. The seasonal variations in temperature and salinity had effect on growth of *G. edulis*. In addition the characteristics of habitat and water current may affect the seaweed growth rates at each station.

SULFATED POLYSACCHARIDES FROM *Caulerpa lentillifera* OF THAILAND

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The *Caulerpa* is a common species in Thailand. A total of twelve species of this taxon has been reportedly found in several parts of the country, along both the coast of the Andaman Sea and the Gulf of Thailand (Lewmanomont and Ogawa, 1995). The *Caulerpa* species is exploited commercially harvested only from naturally occurring populations. Currently, *Caulerpa lentillifera* is used as a biofilter for effluent pond aquaculture, i.e. as an efficient recover of large amounts of dissolved inorganic nitrogen from effluent.

Caulerpa species is known to produce anesthetic toxins such as caulerpin and caulerpicin, which are destroyed upon boiling. The green alga has been found to contain sulfated heteropolysaccharides whose chemical properties resemble fucoidan (Nisizawa, 2002). It is only in recent times that studies have been carried out to determine the characteristics of these compounds. Most water-soluble sulfated polysaccharides of the algae shows anticoagulant activities and some activities were mostly higher than heparin (Nisizawa, 2002). A β -D-glucan has been reported in *Caulerpa simpliciuscula* (Hawthorne et al. 1979). A water-soluble mixture of polysaccharides extracted from *C. filiformis*, is fractionated into an amylopectin type glucan, and sulfated polysaccharide containing galactose, mannose, and xylose. Isolated sulfated polysaccharides from *Caulerpa lentillifera* gathered from Phetchaburi Aquaculture Station, was analysed for the polysaccharide components by TLC. The isolated polysaccharides yielded 0.46 % dw/fw. and contained carbohydrate ca 26.67%. The *C. lentillifera* consisted mainly of galactose and glucose, but no rhamnose and mannose. A similar result was also reported for the acid-extracted polysaccharide from *Caulerpa taxifolia* (Prasada and Venkata, 1986). In addition, the hydrolyzed polysaccharides of *C. lentillifera* showed a fraction of glucan similar to a report of *C. taxifolia* from India (Prasada and Venkata, 1986). A preliminary test for physiological activity of the sulfated polysaccharides showed non-antibacterial and non-anti yeast substance but this is inconclusive since crude polysaccharides samples were used in the analysis. To date no sulfated heteropolysaccharides from the Thai *Caulerpa* species have been investigated chemically.

ON THE *Gracilaria coronopifolia* J. AGARDH (RHODOPHYTA, GIGARTINALES) FROM TOKYO BAY JAPAN

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The *Gracilaria coronopifolia* were found abundantly at the big tidal pool in the entrance of Tokyo Bay, Japan in 1992, were 15-25 cm tall, with infrequently compressed branches 1.0-2.0 mm diameter. The plants were later transplanted to the small inlet. The plants grew well with floating conditions in the inlet for two years. A few cases of *Gracilaria* growing well throughout the year in Japan under the natural conditions have been reported. The distribution this species in Japan is very limited. *G. coronopifolia* were found to grow well only in the Okinawa prefecture, Southern Japan. They grow in groups, on rocks or crevices in the lower intertidal zone exposed to full surf. The plants are found in Tokyo Bay (about 1000 mile from Okinawa) due possibility to distribution by ship although the origin of plants are difficult to confirmed. It is also possible the plants originated not from Okinawa since there are many ships came to Tokyo Bay from outside of Japan.

Observations on the daily growth rates and agar quality of *G. coronopifolia* in Tosa Bay, have been done to asses the feasibility cultivation this species in Southern Japan. In field cultivation experiments, this species had growth rate of $4.2\% \pm 0.4$ in a salinity of 33 ‰. These plants grew well in temperature range from 26 to 31 °C, salinity 30 to 40 ‰ and photon fluencies 85 to 125 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ under the closed-recirculation system (aquatron) experiments. The seaweeds from this study had agar yield of 21.0 % and gel strength of 652 g/ cm².

**MICROARRAY SCREENING FOR WSSV IMMEDIATE EARLY GENES IN
CYCLOHEXIMIDE-TREATED SHRIMP**

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We describe for the first time cycloheximide (CHX)-insensitive genes of shrimp white spot syndrome virus (WSSV). Despite the lack of any shrimp cell line and the difficulty of using CHX *in vivo*, we successfully used CHX as an inhibitor to block *de novo* viral protein synthesis. A global analysis microarray technique was then used to determine kinetic classes, and sixty putative IE (immediate early) genes were identified. Further testing using RT-PCR showed that only 3 of these candidate genes were consistently CHX-insensitive. In a promoter activity assay in Sf9 insect cells using EGFP as a reporter, both the 1k and 2k promoter sequences of one of these three candidate genes (WSSV ORF126) showed very strong promoter activity, producing higher EGFP signals than insect virus OpMNPV IE2 gene promoter. A similar result was also found using Western blots. We therefore designated WSSV ORF126 as WSSV *ie1* (immediate early gene # 1). Although plasmids containing the promoters of the other two candidate IE genes gave negative results in the Sf9 insect cells, these candidates may be shrimp-cell specific and should therefore also be assayed in shrimp cells before being rule out as IE genes.

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Addendum

VIRAL INFECTIONS IN SHRIMP CULTURE PONDS OF COASTAL ANDHRA PRADESH THE PRESENT SCENARIO

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Shrimp culture in India, especially in Andhra Pradesh is facing severe loss due to the outbreak of various viral diseases. White spot syndrome virus is a major threat in India and many other viruses like Monodon baculovirus, Hepatopancreatic parvovirus, Yellow head virus and Reo-like virus are the emerging diseases and showing their influence in culture systems. Incidence of co-occurrence of two or more viruses in a single shrimp from culture pond has also been recorded. The diseased shrimps were collected from shrimp culture ponds of Visakhapatnam, Kakinada and Amalapuram areas of coastal Andhra Pradesh and were analysed for histopathological and PCR studies. The histopathological studies revealed presence of Cowdry A type basophilic inclusion bodies with chromatin margination in the infected tissues which is the characteristic feature of WSSV infection. Presence of MBV occlusion bodies and HPV basophilic intranuclear inclusions with crescent shaped nucleoli which they have pushed to one side of the nucleus were observed in the infected tissues. Densely stained basophilic inclusion bodies typical of YHV were also observed in gill tissues of infected shrimps. 100% of the diseased shrimps collected during the investigation showed WSSV infection where as 40% of shrimps were with WSSV, MBV infections. 25% of the shrimps showed infections with WSSV, YHV & MBV, and HPV and RLV are of rare occurrence. PCR studies revealed the presence of WSSV in all the diseased shrimps suspected to be affected by viral infections. It is suspected in future the shrimp culture system will face problems not only from WSSV but also from other viruses if proper precautionary measures are not implemented immediately.

ASSESSMENT OF FIVE STOCKS OF *Oreochromis niloticus* IN MALAYSIA USING 11 MICROSATELLITE MARKERS

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Oreochromis niloticus, commonly known as tilapia is one of the prime species of interest in the aquaculture industry. However, these genetic resources of tilapia have been poorly managed. Primary introductions of wild stocks frequently consisted of small number of individuals. These were serially distributed so that genetic problems have been passed from farm to farm. In line with the development of molecular markers, polymorphic microsatellite markers were used to assess the population structure for future breeding program. 11 polymorphic microsatellite loci showed significant departures from Hardy Weinberg equilibrium. The results showed that all loci are polymorphic. A dendrogram was constructed based on genetic distances obtained from the gene frequency data indicated the phenetic relationships among eight populations of *O. niloticus*. The genetic distance showed a clear separation between *O. niloticus* and *O. mossambicus*. The mean heterozygosity ranged from 0.6280 to 0.7040 for each population. These markers are indeed useful for identification and resolving the closely related populations.

REINFORCEMENT FOR PULLING OUT RESISTANCE OF THE SELF EXPENDABLE PILE ANCHOR

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Greater holding force of an anchor is required for keeping the position of a large aquaculturing cages. According to the series of model tests of pile anchors with movable fluke, the self expandable pile anchor was developed for a type of reinforced anchor. The result of model test was shown as followings.

Table 1 Comparison of pulling force with pile anchor's weight

Model type	Weight (kgf)	Pulling force (kgf)	Pulling force ratio to weight
Symmetric circular pile anchor	0.16	7.68	48.0
Unsymmetric circular pile anchor	0.18	9.05	50.3
Self expandable pile anchor	0.10	10.21	102.1

It shows about 6 times for symmetric circular pile anchor and 8 times for square pile anchor and unsymmetric circular pile anchor. When compared with its weight, especially the resistance of the square type pile anchor shows about 100 times. Compared with the ordinary pile anchor shown about 50-60 times of its weight, the square type pile anchor shows excellent performance. This self expandable anchor is patented in Korea as Patent No. 0387375. This self expandable pile anchor is expected to be used for the reinforcement of a larger aquacultural netting system.

EFFECTS OF DIETS ENRICHED WITH VITAMIN OR SPIRULINA ON THE GONADAL DEVELOPMENT OF MALAYSIAN MAHSEER *TOR TAMBROIDES* (BLEEKER)

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Information concerning feed and its effect on gonad development is important in broodstock management. For this purpose, a study was carried out on kelah, *Tor tambroides*, a species of endangered recreational fish, as the catalyst to the restocking programme. A study on the effects of diets enriched with vitamin and *Spirulina* towards the growth and development of gonad was carried out using histological observation.

Fish fed diets with *Spirulina* showed higher significant differences ($P < 0.05$) for the Gonadosomatic Index (GSI) compared to fish fed other diets. However, there were no significant differences ($P > 0.05$) shown in the GSI of fish fed diets with Vitamin C, Vitamin E or the control diet. Through macroscopic observation and the histology of the gonad, it was shown that diets with *Spirulina* can stimulate the development stages of the testis and ovary of *T. tambroides* faster than other diets. There were no significant differences ($P > 0.05$) shown between diets with Vitamin C, Vitamin E and the control diet in which the testis and ovaries of fish fed these diets were still immature. Histological result also showed that *T. tambroides* are asynchronous breeders.

In conclusion, the study indicated that *Tor tambroides* can be successfully grown in indoor culture conditions on dry feeds with *Spirulina* as an additive and subsequently released to enhance the wild stock populations.

CRYOGENIC FISH SPERM BANK IN ASIA: THE ROLE OF AQUACULTURE PROGRAM AT THE ASIAN INSTITUTE OF TECHNOLOGY (AIT)

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Genetic preservation of aquatic biodiversity in Asia is critically important. Aquatic systems represent a vast, and largely undocumented reservoir of biodiversity. Many indigenous fish species, including a number that have yet to even be described, are now under threat from several sources including hydropower development, irrigation, land reclamation for agriculture and industrial development, human settlement, pollution and overfishing. Indigenous species representing opportunity as prime candidate for aquaculture are threatened. Additionally, a number of cultured stocks are contaminated through improper broodstock management practices (cross breeding, hybridization etc.). The loss of genetic purity and subsequent inbreeding has been observed in many hatchery propagated stocks. It is thus urgent that efforts are made to establish functional gene banks for these fish species before too much of these stocks are either contaminated or all together lost.

Aquaculture and Aquatic Resources Management Program of the Asian Institute of Technology has been working in many parts of Asia to assist in development of cryogenic sperm banks for many threatened indigenous species. Currently we are actively involved in Bangladesh, Vietnam and Thailand to assist in development of research issues in cryopreservation of aquatic species and formulation of functional cryogenic sperm banks for the purpose of conservation and aquaculture production. Information on the success of cryopreservation of Indian and Chinese major carps (in Bangladesh) using simple protocols, indigenous common carps & Chinese carps (in Vietnam), and threatened Pangasiid catfishes (in Thailand) have been obtained. Some of the results from these countries will be presented and discussed.

Table: Fish sperm cryopreservation program in selected countries of Asia

Country	Species focus	Purpose	Relative success
Thailand	<i>Pangasious hypophthalmus</i>	Model/ Aquaculture	Yes
	<i>P. gigas</i> (endangered)	Conservation/Aquaculture	Variable
	<i>P. larnaudii</i> (threatened)	Conservation/ Aquaculture	Yes
	<i>P. sanitwongsi</i> (threatened)	Conservation/Aquaculture	Incomplete
Vietnam	Several strains of <i>Cyprinus carpio</i>	Conservation	Yes
	<i>Spinibarbus denticulatus</i>	Conservation/Aquaculture	Variable
	Grass carp	Aquaculture	Yes
Bangladesh	Rohita (rohu)	Aquaculture	Yes
	Mrigal	Aquaculture	Yes
	Bighead carp	Aquaculture	No
	Silver carp	Aquaculture	Variable